FINAL REPORT

MAXIMIZING SAMPLING EFFICIENCY AND MINIMIZING UNCERTAINTY IN PRESENCE/ABSENCE CLASSIFICATION OF RARE SALAMANDER POPULATIONS

SERDP Project SI-1393

October 2008

Dr. Mark S. Bevelhimer
Oak Ridge National Laboratory

Mr. Neil R. Giffen
Oak Ridge National Laboratory

Mr. William R. Fields
North Carolina State University

Dr. William W. Hargrove
US Forest Service

SERDP
Strategic Environmental Research and Development Program

REPORT DOCUMENTATION PAGE	*Form Approved OMB No. 0704-0188*

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
31-10-2008	Final	Dec 2004 - Oct 2008

4. TITLE AND SUBTITLE
Maximizing Sampling Efficiency and Minimizing Uncertainty in Presence/Absence Classification of Rare Salamander Populations

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER
2297-S816-S1 (ORNL), SI-1393 (SERDP)

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHOR(S)
Dr. Mark S. Bevelhimer - ORNL
Neil R. Giffen - ORNL
William R. Fields - North Carolina State University
Dr. William W. Hargrove - US Forest Service

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Oak Ridge National Laboratory
Environmental Sciences Division, Oak Ridge National Laboratory, PO Box 2008, Oak Ridge, TN 37831

8. PERFORMING ORGANIZATION REPORT NUMBER
ORNL/TM-2008/192

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Strategic Environmental Research and Development Program
Dr. John Hall
901 N. Stuart St., suite 203
Arlington, VA 22203

10. SPONSOR/MONITOR'S ACRONYM(S)
SERDP

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
SI-1393

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Effective sampling of pond-dwelling larval stages of the federally-listed flatwoods salamander (Ambystoma cingulatum) requires sufficient knowledge of when larvae are present and how best to sample them. The primary objective of this study was to find ways to maximize field sampling efficiency of flatwoods salamanders and minimize the uncertainty associated with declaring absence after repeated non-detects. The project combined results from field sampling at Fort Stewart, Georgia, and two sites in Florida with statistical and simulation modeling approaches. Habitat modeling revealed certain vegetation types that were most often associated with occupancy, although the model developed for the site in Georgia was a poor predictor for Florida sites. We found that estimates of probability of detection are crucial to estimating how many unsuccessful surveys are necessary to conclude that a site is unoccupied with a known level of confidence. Simulations showed that fewer samples are required when occupancy is randomly distributed than when clustered. Knowledge of factors that result in clustered distributions makes adaptive sampling a better option.

15. SUBJECT TERMS
rare species, flatwoods salamander, occupancy, detection probability, Fort Stewart, habitat model

16. SECURITY CLASSIFICATION OF: a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
U	U	U	UU	165	Mark Bevelhimer
					19b. TELEPHONE NUMBER *(Include area code)* 865-576-0266

CONTENTS

CONTENTS (Cont'd)

CONTENTS (Cont'd)

Page

CONTENTS (Cont'd)

Page

LIST OF FIGURES

Page

LIST OF FIGURES (Cont'd)

Page

LIST OF FIGURES (Cont'd)

Page

LIST OF TABLES

Page

LIST OF TABLES (Cont'd)

Page

LIST OF ACRONYMS

AFB	Air Force Base
AIC	Akaike Information Criterion
ANF	Apalachicola National Forest
EAFB	Eglin Air Force Base
FFWCC	Florida Fish and Wildlife Conservation Commission
GIS	Geographic Information System
NF	National Forest
NWI	National Wetlands Inventory
NWR	National Wildlife Refuge
SMNWR	Saint Marks National Wildlife Refuge
SVL	Snout-vent length
TL	Total length
USFWS	United States Fish and Wildlife Service

ACKNOWLEDGEMENTS

The authors thank the Strategic Environmental Research and Development Program (SERDP) for the financial assistance that enabled us to undertake this project. Appreciation for technical assistance is extended to Mr. Bradley Smith, Executive Director and Drs. Robert Holst and John Hall, Sustainable Infrastructure Program Managers, former and present, and to the HydroGeoLogic, Inc., staff for their administrative assistance. This research was performed at Fort Stewart Military Installation and Oak Ridge National Laboratory. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract DE-AC05-00OR22725. We appreciate the support of Tim Beaty, Larry Carlile, Stella Osborn, Dena Thompson, and John Macey of the Fort Stewart Wildlife Branch Office throughout all aspects of this study. Fort Stewart Range Control staff kindly assisted with access issues. We thank Kara Ravenscroft and Mike Ravenscroft for their diligent assistance in the field. We are indebted to several people who helped us gain access and collect data at sites in Florida including Amanda Stevens and Sarah Eddie at Jackson Guard (Eglin Natural Resources headquarters) – Eglin Air Force Base; David Printiss (The Nature Conservancy) and Renee Ripley (US Forest Service) – Apalachicola National Forest; Joe Reinman – St Marks National Wildlife Refuge; Kevin Enge (Florida Fish and Wildlife Conservation Commission); Hildreth Cooper (US Fish and Wildlife Service); and John Palis. We thank Stephanie Floyd and Allison Fortner of Oak Ridge National Laboratory for assistance in preparation of the final report.

EXECUTIVE SUMMARY

Effective sampling of pond-dwelling larval stages of the federally-listed flatwoods salamander (*Ambystoma cingulatum*) requires sufficient knowledge of when larvae are present and how best to sample them. The primary objective of this study was to maximize field sampling efficiency of flatwoods salamanders and minimize the uncertainty associated with declaring absence after repeated non-detects. Our approach was to evaluate sampling method efficiency, the relationship between pond habitat and larvae residency, and the effect of sample design on sampling success.

FIELD SAMPLING

We surveyed salamander larvae and pond habitat at Fort Stewart military installation for three years and at several locations in Florida on a limited basis. Our studies on method effectiveness demonstrated that the most time efficient and cost effective sample method is dipnetting. Larvae were detected at one pond at Fort Stewart in 3 of 4 years of the study, but not at 59 other ponds that were sampled. These detections represent the only observations of successful breeding in a natural wetland in Georgia since 1999. Our inability to find larvae in more than one pond at Fort Stewart confirms what appears, based on historic capture records, to be a significant decline in flatwoods salamander abundance at Fort Stewart. To better predict annual variation in pond residency, we developed a model that uses rainfall data and likely growth rates to predict hatching dates and period of pond residency. With two years of data that likely represent the extremes of pond residency in Georgia we found that date of hatching can occur as early as the first week of January and as late as the first week of March and, that in some years, larvae may be found in the pond as late as the end of May. We recommend that the most opportune time of year to sample flatwoods salamander larvae is during the second and third months after a pond fills to at least half, typically sometime within February to April. Using removal study data from temporary enclosures at St Marks NWR we derived a relationship between larval density and capture rate that allows future estimates of larval density based on observed capture rates. The proportion of total larvae present that were captured during a pass averaged about 40% (range of 22-65%) and was not dependent on larval density.

HABITAT ASSESSMENT

We used statistical models to predict larval occupancy of wetlands and to quantify the similarity among wetlands based upon habitat data from pond-specific vegetation and landscape data. The presence of flatwoods salamanders was positively associated with a native iris species and the presence of facultative and obligate wetland plants, and increased canopy cover was strongly associated with unsuitable wetlands. Landscape structure was also an important predictor of habitat use. For example, including distance from streams improved prediction of habitat in regression analyses, and discriminant function analyses with landscape data had a higher rate of correctly classifying known breeding sites as being occupied. Models developed for Fort Stewart populations did a poor job of predicting presence for sites at St Marks NWR and Apalachicola NF. Results from hierarchical clustering indicate that this may be due to differences in wetland vegetation and landscape structure among the sites in Florida and Georgia. Developing habitat models for rare or declining species is particularly challenging because a species may decline for

reasons that are not directly related to habitat structure, and if populations become restricted in their distribution or shift habitat associations as a result of other ecological factors such as disease, predation, and competition, then this may cause habitat models to perform poorly. Amphibians are also challenging subjects for habitat modeling because their occupancy in ponds is highly variable through time. Because the habitat modeling did not identify the habitat of the historically occupied ponds at Fort Stewart as particularly unique, we believe that either 1) there are many ponds at Fort Stewart that are capable of supporting flatwoods salamander breeding or 2) the features of the habitat that we used as input variables to the models do not include all the important features.

STOPPING RULES ANALYSIS

Estimating the probability of detection is crucial to being able to calculate how many unsuccessful surveys are necessary to conclude that a site is unoccupied with a known level of confidence (e.g., 95%). We developed two methods using the larval survey data to estimate probability of detection, which is a key component for calculating sampling effort needed to be confident that an undetected pond is actually unoccupied. The first method uses detection success rate for 5-min survey intervals and the second uses per-minute capture rate. With either method we can relate probability of detection to capture rate and, based on our enclosure study results, also to larval density. These relationships allow us to base calculations of number of necessary surveys on an expected larval density instead of a detection probability, a concept that for most biologists has less meaning and utility than population density. Therefore, when an estimate of detection probability for a pond is unavailable or unattainable, biologists have two options if they want to determine how many surveys are necessary. They can either 1) select a minimum density above which they want to be reasonably sure larvae do not exist and calculate the required number of non-detects necessary to reach that conclusion, or 2) select a level of confidence with which they are comfortable and, knowing that their sampling effort is limited to a set number of surveys, they can calculate the density above which they are reasonably certain that larvae do not exist.

We developed a computer model to simulate periodic sampling of ponds for flatwoods salamander larvae. The model was used to determine the effectiveness of random versus adaptive sampling and the effect of stopping rules on survey success. Stopping rules for the purpose of this study refer to the number of times a pond is sampled without finding a larval salamander before sampling should be ceased because either the probability of the pond being occupied is extremely low or because distributing the effort to other ponds increase the probability of detecting larvae elsewhere. We found that neither sampling mode (random versus adaptive) was better than the other in all circumstances. In general, when occupied ponds were distributed in clusters, adaptive sampling based on pond proximity produced more detections than random sampling. In fact, if surveyors are able to identify any factor that is correlated with salamander presence, then adaptive sampling with that factor as a basis will produce the most efficient sampling design. When occupied ponds were randomly distributed across the pondscape, random sampling was slightly better. With regards to stopping rules, when adaptive sampling is justified, stopping rules of four or five produce more detections, but when surveyors have no basis for adaptive sampling, random sampling with a stopping rule of one or two trips is better or equal to

longer stopping rules. The model was coded in the R, a programming language which is widely used and available at no cost.

TECH TRANSFER

The results (including model code) of this study will be distributed to biologists and resource managers that are involved in flatwoods salamander conservation in the southeastern U.S. A workshop was held at the conclusion of the study to brief regional biologists and resource managers on the findings of the study. Specific recommendations are presented to direct future monitoring of flatwoods salamanders at Fort Stewart with regard to how and when to sample, which ponds to sample, and how to determine when enough sampling has been completed after repeated non-detects. The methods developed here are also applicable to the detection of other rare species including pests and exotic invaders.

1. OBJECTIVES

The primary objective of this project was to evaluate and develop methods and tools to improve the detection of a rare salamander and reduce the uncertainty in classifying a habitat as unoccupied when none are found.

Secondary objectives included:

- test and when possible improve the success and efficiency of field sampling techniques,
- determine capture and detection rates for salamander larvae at different densities,
- identify the characteristics of suitable habitat and evaluate the suitability of ponds of unknown occupancy,
- evaluate possible effects of base activities on salamander populations,
- determine the number of unsuccessful surveys necessary to claim absence with an acceptable level of confidence, and
- evaluate the relative effectiveness of different sampling modes and designs, with particular emphasis on stopping rules to minimize wasted effort and maximize confidence.

2. PROJECT BACKGROUND

Ambystoma cingulatum Cope (flatwoods salamander) was listed as federally threatened in 1999 due to range-wide population declines attributable to habitat loss and habitat conversion for silviculture, agriculture, and residential and commercial development (U.S. Fish and Wildlife Service [USFWS] 1999). Restricted to northern Florida and the Coastal Plain of South Carolina, Georgia, and Alabama (Figure 1), this species is endemic to mesic flatwoods and savannahs dominated by *Pinus palustris* Mill (longleaf pine) – *Aristida stricta* Michx (wiregrass) where it breeds in small (1.5 ha mean size), isolated depressional wetlands (Palis 1997). Wetlands used by breeding flatwoods salamander are ephemeral and usually dry on an annual basis (Anderson and Williamson 1976, Palis 1997). The basins of breeding ponds are usually abundantly vegetated with graminaceous vegetation and are often partially forested with *Taxodium ascendens* Brongn (pond cypress), *Nyssa sylvatica* Marsh (black gum), and *Ilex myrtifolia* Walt (myrtle-leaf holly) (Jensen 1999; Palis 1996, 1997).

A recent molecular and morphological analysis (Pauly et al. 2007) suggests that what are presently recognized as flatwoods salamanders should instead be classified as two distinct taxa. Based on mitochondrial DNA, morphology, and allozymes, they propose that populations east of the Apalachicola River drainage should be referred to as *A. cingulatum* and populations to the west as *A. bishopi*. Except for 1 week of sampling at Eglin Air Force Base, we sampled populations east of the Apalachicola River drainage. Although this proposed division in classification appears to be generally accepted by the herpetological community, we will refer to all as flatwoods salamander throughout this report.

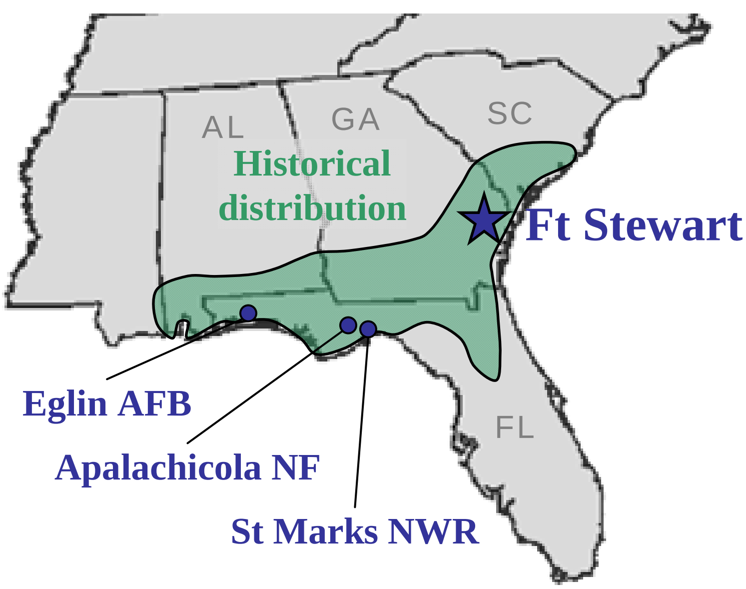


Fig. 1. Flatwoods salamander historical distribution.

During the nonbreeding season, adult flatwoods salamanders are fossorial and inhabit crayfish burrows and other ground cavities within mesic flatwoods and savannah habitats located near breeding ponds (Palis 1996). At the time when adults migrate to breeding sites (mid-October – mid-December), the basins of breeding ponds are typically dry (Anderson and Williamson 1976, Palis 1997, D. Stevenson, unpubl. data). Females deposit eggs terrestrially in moist microhabitats (e.g., at entrances to crayfish burrows or under sphagnum moss, leaf litter, or dead grass) (Anderson and Williamson 1976). *Ambystoma opacum* Gravenhorst (marbled salamander), also a fall breeder, is the only other ambystomatid salamander species that deposits eggs terrestrially (Petranka 1998). Flatwoods salamander eggs begin to develop immediately after they are laid but do not hatch until inundated by rising pond levels, which might occur weeks or even months after deposition (Anderson and Williamson 1976; Palis 1995, 1997), typically December to February. The aquatic larvae of flatwoods salamanders inhabit a specific microhabitat – graminaceous vegetation of linear growth form (Palis 1996, Sekerak et al. 1996) – that is likely maintained by occasional fires burning into or through the dry pond basins (Bishop and Haas 2005, Palis 1997, Sekerak et al. 1996). Larval development is completed in 11 to 18 weeks and timing of metamorphosis may be influenced by pond drying (Palis 1995). More detailed life history and ecology information can be found in USFWS (1999) and Palis (1996, 1997).

The current distribution of flatwoods salamander is mostly restricted to large tracts of public lands, such as national forests, and military bases. One such sanctuary is Fort Stewart military installation, which is home to many rare and imperiled species of reptiles and amphibians (Stevenson 1999, USFWS 1999). Herpetofaunal inventories conducted at Fort Stewart during the mid to late 1970s by the Savannah Science Museum (Williamson and Moulis 1979), The Nature Conservancy from 1992-1995 (Gawin et al. 1995), and the Fort Stewart Wildlife Branch Office from 1996 to present confirmed flatwoods salamander breeding on at least one occasion at 25 ponds (henceforth referred to as “known” ponds).

The presence of breeding adults, eggs, and larval salamanders at a pond in any particular year is a function of a variety of factors (Figure 2). For adults, appropriate terrestrial upland habitat must be near the pond, generally within a few hundred meters (USFWS 1999). The habitat must include the appropriate vegetative cover and soil type and usually includes other animals (e.g. crayfish) that provide burrows used by flatwoods salamanders. Migration corridors must be accessible when adults are cued to move to the ponds by autumn rains. For eggs and larvae, the most critical factor is winter rains that submerge the eggs, allowing them to hatch and the larvae to rear. Natural pond hydroperiods (i.e., ponds flooding in winter, holding water through April, and then drying later in the year) are necessary for successful reproduction and juvenile rearing. Water quality, in-pond habitat, and food resources are also crucial, though these requirements are not well understood. Because larval salamanders are susceptible to predation (particularly by many fish species), flatwoods salamanders are rarely found in permanent ponds, which typically support a variety of aquatic predators.

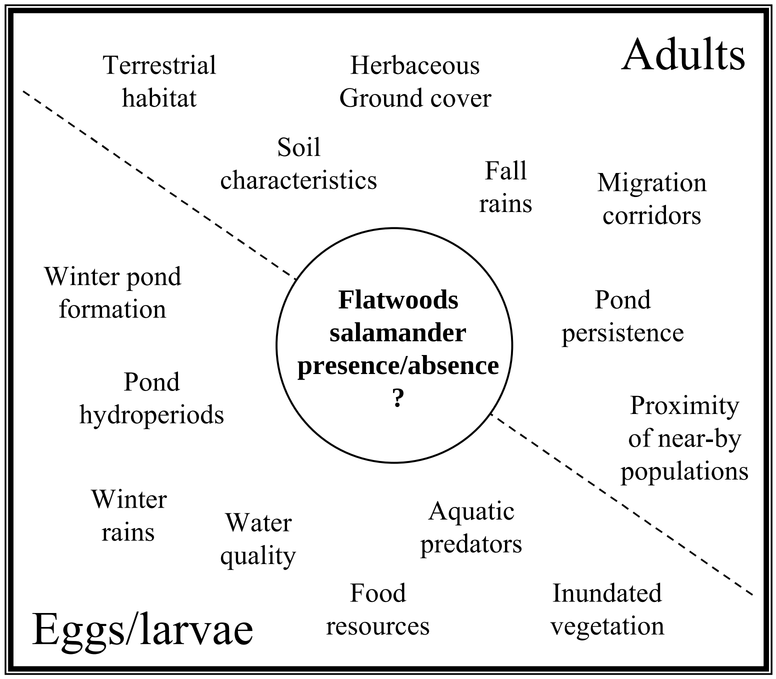


Fig. 2. Factors that affect the presence of flatwoods salamander eggs, larvae, and adults in Fort Stewart ponds.

Of 1,364 seasonal ponds on Fort Stewart, 483 have been identified through preliminary screening as potentially supporting flatwoods salamander populations. From 1992-1995, about 100 of these ponds were sampled, and larvae were found in 20 of them. Breeding has been confirmed at two other sites (one a gravel borrow pit) in other years bringing the total number of confirmed breeding ponds at Fort Stewart to 22. Most of these ponds are located in the southeastern and northwestern quarters of the base although the small number of previously detected ponds in some areas of the base could be a function of limited access to surveyors due to military training (Figure 3). Biologists at Fort Stewart base their annual monitoring design on sampling requirements included in Fort Stewart's Integrated Natural Resources Management Plan: Multi-species Endangered Species Management Plan (Appendix A.1). The plan states that 10 of the 'known' ponds where breeding has been documented in the past are to be sampled annually during February and March. Eleven other confirmed breeding ponds are to be sampled biennially and other potential habitat (which includes unnatural features such as borrow pits and fire break ditches) are to be sampled when possible.

Classification of presence or absence of flatwoods salamanders at these ponds may impact the types of activities (e.g., training exercises, facility development, timber harvest, road construction and road use) that can take place in their watersheds. Resource managers at Fort Stewart currently follow USFWS guidelines regarding restricted timber harvest within primary (164 m) and secondary (450 m) buffer zones surrounding known breeding sites of flatwoods salamanders. For reference, Figure 3 indicates the area included in a 500 m buffer zone around the known ponds at Fort Stewart.

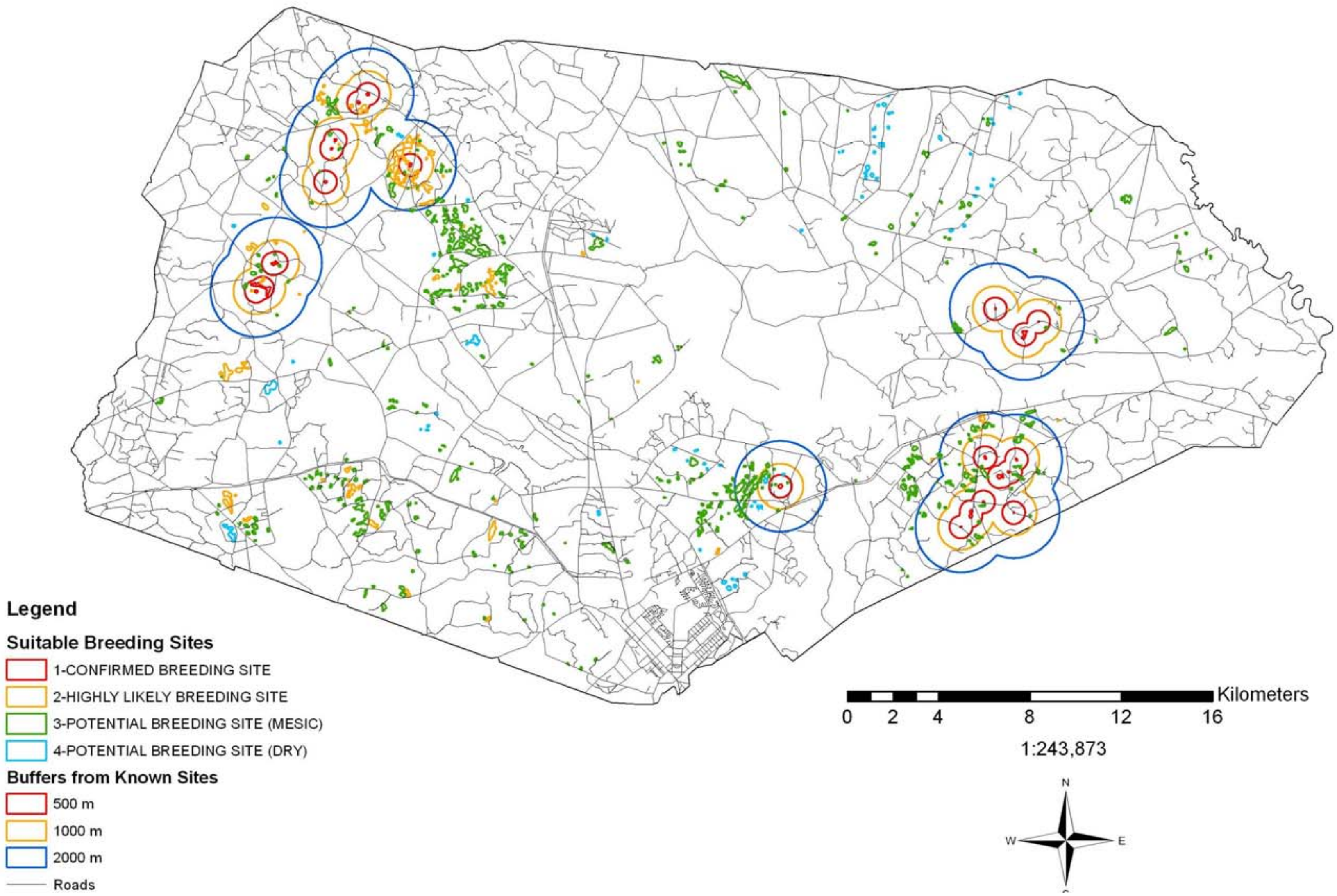


Fig. 3. Location of ponds known to have historically supported flatwoods salamander breeding at Fort Stewart.

Determining which ponds are used by flatwoods salamanders for breeding is a difficult task. When an individual larval flatwoods salamander is found during a pond survey, classifying the pond as a breeding site can be made with 100% certainty. However, concluding that the species is absent with high certainty is not as straightforward. For rare species, failure to detect individuals at a site even after repeated sampling does not necessarily equate to their absence. The likelihood of collecting at least one flatwoods salamander in a pond where they are present depends on the density of individuals, the efficiency of the sampling gear, and the amount of sampling effort. Likewise, the certainty with which one can classify absence after repeated unsuccessful sampling is a function of many factors, including the amount of effort without capturing a specimen and the probability of detecting an individual if they are present, given sampling gear efficiency. If the rate of inhabitance for all 483 ponds is similar to those ponds already sampled (roughly 20 out of 100), then we can expect that for about 80% of the ponds a decision will have to be made about the appropriate time to quit sampling and conclude that flatwoods salamanders are not present.

Sampling over 400 ponds at Fort Stewart to assess the presence of flatwoods salamanders is a daunting task that will take several years and a great deal of effort to complete. Most ponds will have to be sampled repeatedly before a decision can be made. Being able to concentrate efforts on those ponds that are most likely to support flatwoods salamander breeding and being able to select the most effective sampling methods would significantly minimize effort and cost. Knowing when to quit sampling after not finding a specimen after repeated sampling would also minimize the effort expended on ponds that are uninhabited. We completed a series of inter-related tasks to address these issues so that surveys for flatwoods salamanders can be completed both effectively and efficiently so that decisions about flatwoods salamander presence and absence are made with high confidence.

The problem of knowing whether rare species are present is common across many military installations and other government lands. It has also been an issue for years in agricultural pest control (Wald 1947) and various conservation efforts (Green and Young 1993, Reed 1996, Kery 2002). The decision to protect a crop against a damaging pest when detected, or to protect a threatened species when detected are both decisions with significant (and potentially costly) consequences.

Although we were primarily interested in improving sampling efficiency for a threatened salamander, the study was pursued with the intent to focus on approaches that have general applicability for other taxa. The approaches we used in this study (i.e., simulated sampling with stopping rules and habitat modeling) to reduce the amount of sampling necessary to obtain reliable results are not specific to flatwoods salamanders. Likewise, the challenges of determining detectability (or capture probability) are faced by biologists dealing with a wide array of species.

3. TECHNICAL APPROACH

The scope of this study consists of four tasks (three research and one tech transfer) that in combination address the problem of maximizing field sampling efficiency while minimizing the uncertainty associated with characterizing the presence or absence of a rare organism (specifically flatwoods salamanders) at a particular location. Each task is briefly described below and described in full in chapters 4 through 7. Each research task chapter includes background, methods, results, and discussion sections.

3.1 TASK 1. FIELD COLLECTION AND METHOD COMPARISON

Field collection and experimentation were used to investigate the level of effort necessary to detect larval flatwoods salamanders and to compare capture efficiency of sampling methods used in the collection of larval flatwoods salamanders. The sampling effectiveness of different methods and method deployment was determined through systematic sampling in known breeding ponds throughout the rearing season (Feb-Apr). Efficiency of the different methods was also calculated on a cost basis (i.e., investigator effort and materials costs) to determine the cost efficiency of each method. Because salamander abundance is an important factor in detectability, larval abundance was estimated at several sites to derive a relationship between catch rate and larval density for different sampling methods. A key to successful detection of flatwoods salamanders is sampling at the appropriate time of the year. Data on larvae captured during this study and in prior monitoring at Fort Stewart were used to better understand the relationship between period of pond residency and seasonal precipitation. This task is described in Chapter 4.

3.2 TASK 2. HABITAT MODEL

Limited resources often prevent biologists from exhaustively sampling all available habitat to the point of characterizing a species absence with near certainty. Instead, sampling effort is usually prioritized such that sites or habitats that are most likely to host a target organism are searched first or most often. Quality habitat is one of the key features used in such prioritization. In this task, we developed several habitat models to identify those ponds with characteristics most like ponds already known to support flatwoods salamander reproduction. For habitat characterization data we used 1) a past assessment by local flatwoods salamander experts, 2) physical data from individual ponds and their watersheds collected by our team, 3) existing GIS layers and remote sensing data sources, and 4) historic salamander presence/absence data. Habitat data included both biological information (e.g., plant species present) and abiotic data (e.g., proximity to roads or permanent water). Complimentary statistical approaches were used to test hypotheses about factors related to the presence of salamanders, predict whether potential breeding sites were occupied, and quantify the variation in wetland habitats. In addition to predicting suitable habitat, the modeling also identifies which features of the habitat are the best predictors of salamander presence. These features could be positive correlates, such as a particular plant species, or negative correlates, such as the presence of man-made obstacle to natural migrations. For example, environmental variables that are directly related to base activities (e.g., the proximity of

existing roads) were specifically evaluated for their effect on salamander presence. The transferability of the model to other populations of flatwoods salamanders was tested by performing model validation with habitat data from Florida sites. This task is described in Chapter 5.

3.3 TASK 3. DEVELOPMENT OF STOPPING RULES

For many monitoring programs, the choice of when to stop sampling a particular habitat (in our case a pond), either in terms of sampling effort per year or number of years of sampling, is largely arbitrary, often resulting in a classification of 'absence' of unknown certainty. The goal of this task is to develop an analysis framework based on statistical probability to determine how much sampling is necessary for a given pond to produce a specified level of confidence in classification and to quantify the level of confidence for any absence classification. Theoretical models based on probability statistics and a simulation model to test various sampling schemes were used to identify the most efficient sampling designs given various underlying circumstances related to salamander abundance and detectability. To perform this analysis, estimates of salamander presence, abundance, and probability of capture are needed; this information will be generated from previous tasks. Based on these results sampling schemes and rules can be recommended to Fort Stewart. This task is described in Chapter 6.

3.4 TASK 4. TECH TRANSFER

Producing results and developing tools that are useful to resource managers working with rare species is an important objective of this study. To ensure that the study objectives addressed the needs of biologists that worked with this species, we hosted a workshop during the first year of the study to discuss our study design with regional biologists. At the conclusion of the study, a follow-up workshop was held to disseminate results and educate others on the use of developed tools. Tech transfer is described in the final discussion in Chapter 7.

4. TASK 1: FIELD SAMPLING FOR FLATWOODS SALAMANDER LARVAE

4.1 INTRODUCTION

Successful management and conservation of flatwoods salamander habitat at Fort Stewart and elsewhere will depend on successfully identifying active and potential breeding sites among hundreds of potential ponds. Although biologists have sampled amphibians for years and have recently observed a marked decline in abundance of many amphibian species throughout the world, there is a general lack of standards and protocols for status assessment (Buech and Egeland 2002). Some studies have been performed to assess the relative effectiveness of different collection methods for certain amphibian species (Buech and Egeland 2002), but no such studies have looked specifically at the capture of flatwoods salamanders.

Both adult and larval flatwoods salamanders are difficult to sample because of their rarity and the habitats they frequent (i.e., underground burrows and densely vegetated ponds). Because the most viable field method for locating adults (monitoring drift fences during adults' migrations to and from breeding sites) is labor intensive, the best way to determine the presence of flatwoods salamanders at a pond is by sampling for larvae with various types of traps and dipnetting (Bishop et al. 2006, Palis 1996). Sampling for larvae is also challenging because they typically reside in dense inundated vegetation that is difficult to sample, densities are often low, and abundance and period of pond residency vary annually and among ponds (Bishop et al. 2006, Palis 1997, Sekerak et al. 1996). Because these methods sample such a small area relative to the entire pond, the probability of capturing a larval flatwoods salamander when they are not abundant is small. Seining is largely ineffective because a large portion of these ponds is comprised of areas that are not inundated much of the year and thus are covered with herbaceous vegetation.

For these reasons it is crucial that biologists use the most effective sampling methodology and clearly understand the factors that affect salamander presence and detection.

The first three objectives of this task were to:

- test various capture techniques for detecting flatwoods salamander larvae and determine their relative effectiveness and the amount of effort and cost to deploy each, and

- sample potential breeding ponds to determine larval encounter and occupancy rates,

- derive a relationship between catch rate and salamander density.

Successful reproduction of flatwoods salamanders is closely linked to the quantity and timing of precipitation throughout the breeding and development period (Oct-Apr). Adults typically move to breeding areas (pond depressions) on rainy fall nights (Oct-Dec) to breed and lay eggs on vegetation in dry ponds. Eggs lay dormant for many weeks or months until inundated by rising water after which they hatch within a day or two. Larval salamanders mature in ponds feeding on

zooplankton till metamorphosis sometime in February to May, depending on when they were hatched. Because the period of pond residency of larval salamanders various annually depending on the timing of precipitation and pond filling, it is not possible to sample at the same time every year and expect the same degree of sampling success. To maximize sampling success it is necessary to be able to predict when hatching occurs so that the ideal sampling period can be identified. Therefore, additional objectives were:

- to evaluate the relationship between hatching date and pond filling, and

- to evaluate survey success at Fort Stewart over the past 15 years relative to sampling success during this study.

Information collected from these tasks will be used to evaluate the effectiveness of various sampling schemes and to make recommendations through our stopping rules model as to the most efficient way to sample flatwoods salamanders.

4.2 MATERIALS AND METHODS

4.2.1 Site Descriptions

Fort Stewart (113,064 ha) is located in the Atlantic Coastal Plain of southeastern Georgia and supports significant areas of intact, fire-maintained longleaf pine ecosystems that contain embedded depressional wetlands (Carlile 1995, Gawin et al. 1995). Within these longleaf pine ecosystems are nearly 500 ponds that have been identified as potential flatwoods salamander breeding habitat. These breeding sites occur in both wet flatwoods and sandhill landscapes. Controlled burning regimes intended to maintain natural habitat for red-cockaded woodpeckers also maintain the natural plant communities in and around the depressional wetlands.

Eglin Air Force Base (187,854 ha) is located on the East Gulf Coastal Plain in the panhandle of Florida. It is the largest forested military reservation in the United States. Much of the base consists of sandhills and long-leaf pine savanna. There is approximately 1,300 kilometers of rivers and streams, 32 lakes, 10,525 ha of other wetlands and 32 kilometers of shoreline on the Gulf of Mexico. The base contains the most significant area of steephead seepage streams in the state of Florida. Eglin supports 67 rare plant species, 34 rare animal species, 11 federally listed threatened and endangered species and 81 Florida State listed threatened and endangered species.

Apalachicola National Forest (228,729 ha), located southwest of Tallahassee, Florida, in the Florida panhandle, was established in 1936 on land that was in poor condition due to bad timber and turpentine producing practices. It is now a diverse forest with the largest red-cockaded woodpecker population in the world. The forest is on relatively flat terrain and is the largest National Forest in Florida. It contains two major rivers, the Ochlockonee and the Sopchoppy. The forest ecosystem includes longleaf pines, wiregrass, savannahs, wetlands and cypress ponds. The area contains flatwoods salamander breeding sites primarily in wet flatwoods and savannah landscapes.

St Marks National Wildlife Refuge (27,125 ha), established in 1931 to provide wintering habitat for migratory birds, is one of the oldest refuges in the National Wildlife Refuge System. It is located in Wakulla, Jefferson and Taylor counties along the Gulf Coast of northwest Florida, approximately 40 kilometers south of Tallahassee. The refuge spans over 69 kilometers of coastline. The topography is relatively flat, with woodlands interspersed with ponds and sawgrass sloughs. Elevations range from open water on Apalachee Bay and barrier beaches to approximately 30 feet above mean sea level. The refuge contains seven rivers and numerous creeks. The climate is moderate, with temperatures ranging from 21 to 96 degrees, and with annual rainfall of 55 inches. Natural salt marshes, tidal flats and freshwater impoundments attract thousands of waterfowl, shorebirds, wading birds and other animals. Most of St. Marks' known flatwoods salamander breeding sites occur in depressional wetlands dominated by sawgrass.

4.2.2 Methods Comparison

Field studies were carried out primarily at Fort Stewart, with supplemental sampling at the three Florida locations (Table 1). We sampled primarily in February and March with some sampling as early as January and as late as June.

Table 1. Time and location of field sampling during study

Year	Location	Sampling period
2005	Fort Stewart	Feb 21 – Jun 3
2006	Fort Stewart	Jan 18 – Mar 20
	Eglin Air Force Base	Mar 14 – Mar 15
2007	Fort Stewart	Jan 15 – Mar 31
	St Marks National Wildlife Refuge	Feb 20 – Feb 22
	Apalachicola National Forest	Feb 19 – 21
2008	Fort Stewart	Feb 27

In year one of the study, our first objective was to test the relative effectiveness of four common larval collection methods: dipnets, metal funnel traps, plastic funnel traps, and wood-framed box traps. Experiments consisted of deploying the selected methods simultaneously in a semi-randomized block design in Fort Stewart ponds where flatwoods salamander breeding had been documented in the past. In 2005 from mid February to late March we sampled intermittently eight known breeding ponds that ranged in size from about 1.3 to 4 ha. Three of the eight ponds were located on the west side of Fort Stewart, and five on the east side about 34 km away – all are located in Liberty County. Within a pond we identified and marked with numbered flagging tape at least 16 sample sites based on a qualitative judgment of what we considered suitable larval habitat with adequate water depth for sampling. These sites were vegetated with moderate to profuse herbaceous cover and had an average water depth of about 15-30 cm.

In 2007 we also conducted a method comparison study at three breeding ponds at St Marks NWR. This study was not as controlled spatially as the Fort Stewart study as we did not use

distinct sampling locations within each pond. Sampling with plastic and metal traps was conducted 2 to 4 days after dipnet sampling.

Dipnetting – Palis (1997) found that dipnetting is an effective way to sample for flatwoods salamanders and describes several sweeping techniques. This method can be performed fairly quickly, depending on how many sweeps are made, and it requires minimal preparation. In this study we used long-handled 40-cm diameter D-shaped dipnets (5-mm mesh). We typically used two to three surveyors covering different locations in a pond, rotating between previously marked sampling points each day. Dipping was conducted by passing the net through the water using a sweeping motion that covered approximately 1 square meter of area for each dip. Vegetation was sometimes agitated by the netter (by foot) prior to dipping. The number of dips conducted by each surveyor per 5-min interval was recorded. Surveyors moved to a new sampling point after each 5-min dipping session. The total dipping effort per point covered several square meters. Generally, four to six sampling locations were covered per pond visit. Captured organisms were counted, identified and immediately released. Captured salamanders were sometimes held for a short period of time in a bucket or submerged netted enclosure for measurement.

Dipnetting can be conducted in very shallow water; however, heavy vegetation and/or detritus, and other obstructions (i.e., sticks, etc.) can hinder the success of this method. Such conditions can lead to nets being snagged during sweeping, unacceptable drag during sweeping and/or excessive debris in the net. This leads to an increase in man-hours required for sampling, and impacts search image due to the need to sift through excessive debris in the net. These difficulties can normally be minimized as the dipnetter gains experience, learns to avoid such obstacles and improves their search image for the target species. Dip nets are light and easily transported, even to remote ponds that require travel long distances on foot. This is a quick and easy method for sampling flatwoods salamander larvae, even where only one surveyor is involved in the sampling.

Funnel Traps – Minnow traps and funnel traps are passive methods that are employed by placing the traps in the ponds and allowing them to sample for several hours, usually overnight. Trap success depends on regular movement of the larvae such that they encounter and enter the trap. Once inside they are not able to find their way out. These methods sample for a longer period than dipnetting without requiring the investigator to be at the location, except for when traps are deployed and again when checked. We deployed commercially-made plastic (4.8-mm mesh) or metal (3.2-mm mesh), double-opening, funnel traps (Figure 4). Traps were placed at a depth or perched on debris such that air-breathing organisms had access to the water surface. Four plastic and four metal traps were generally deployed at each of four sampling locations each sampling day at a particular pond. Traps were rotated to different sampling locations after one trap night, and a total of twelve sampling locations would generally be covered per week in three trap nights.

Use of the cylindrical funnel entrance traps is limited by pond depth, due to the cone-shaped entrances, and will not function in less than about 12 cm of water. These traps are easily stacked within one another for transport; however, the need to transport traps long distances on foot can become burdensome (especially for metal traps that are significantly heavier than the plastic

traps). The need to transport a number of traps for long distances (normally 32 at a time for this study) typically requires participation by a minimum of two surveyors. Trap deployment once at the pond can be completed very rapidly. These traps are easy to assemble and check. Organisms can be quickly identified and released, due to the hinged assemblies that allow for easy opening.

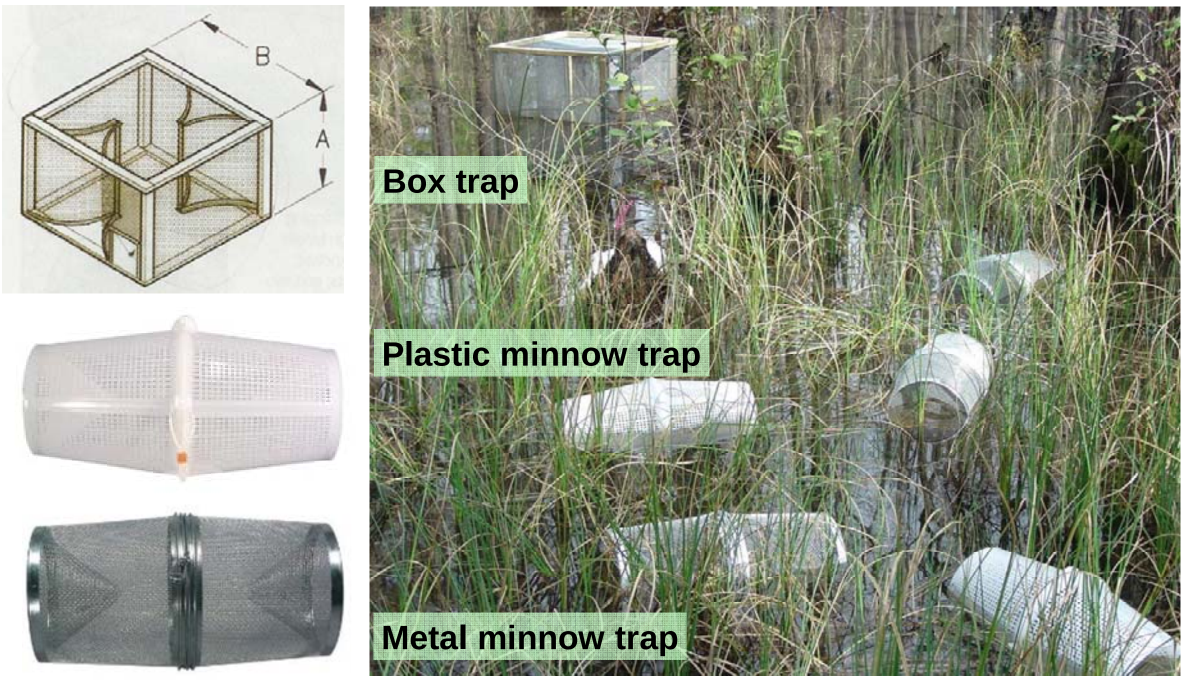


Fig. 4. Three trap types used in methods comparison study.

Box Traps – Mushet et al. (1997) described a funnel-like trap design that samples the entire water column and has been successfully used to sample a different *Ambystoma* species. We used a similarly designed 61 x 61 x 46 cm wood-framed box trap with 2 vertical funnel entrances (3-mm mesh) (Figure 4). Box traps and minnow traps were purchased from Aquatic Eco-systems, Inc., Apopka, Florida. One to two of the box traps were generally deployed along with the plastic and metal funnel traps, placed either with the funnel traps or at a separate location. These traps were also rotated to different sampling locations after one trap night, and a total of three to six locations would generally be covered per week in three trap nights.

These traps have the advantage that the funnel entrances cover the entire water column; therefore, they can be used in very shallow areas. However, the bulkiness of these traps posed difficulties for transport (a typical person can only handle one at a time). Simply moving these traps around to different sampling locations within the pond takes some effort. The large size of these traps also presents difficulties when setting them in ponds with dense vegetation and/or debris. Trap design, with only one small opening to free trapped organisms also had an impact on the man-hours required for trap checking. They are most effective in larger open areas of the pond with few obstructions. Because these traps are so difficult to transport on foot, they are best deployed in ponds with close vehicular access.

Sampling Scheme – Within a pond for a typical day four sites were randomly assigned a standard effort of dipnetting (which was usually 2 surveyors dipnetting for 5 min each); four sites were assigned a combination of 4 plastic and 4 metal minnow traps deployed for 24 hours; and to two sites were assigned a box trap deployed for 24 hours. We rotated dipnetting and trapping efforts daily among the sites within a pond for 2-3 days. For example, during 3 days of sampling at one pond we would typically dipnet a total of 120 minutes (3 days X 4 sites X 2 netters X 5 minutes), trap for 46 trap nights for both metal and plastic funnel traps (3 nights X 4 sites X 4 traps), and 6 box trap nights (3 nights X 2 traps). We counted and measured all flatwoods salamander larvae captured (snout-vent length [SVL] and total length [TL] in mm) and released them shortly after processing to their original site of capture. Most other organisms (amphibians, crayfish, and fish) were counted and identified to species when possible and released immediately to where they were captured. Investigator time in and out of the ponds as well as the time required to perform various tasks was recorded for later estimation of investigator effort for the various methods (Figure 5).

Fig. 5. Research team members check minnow traps in typical Fort Stewart flatwoods salamander habitat.

Method efficiency was measured in standard ecological terms of number of individuals captured per unit of sampling effort (such as hours employed in the case of traps or number of sweeps in the case of dipnets), as well as in terms of the amount of human effort required. For methods that are employed in a similar manner, such as different passive trap types, catch per unit effort in terms of number of flatwoods salamander larvae per hour of sampling (i.e., time trap is in the water) is a useful metric for comparison. However, to compare passive and active methods, a

more useful measure is the number of larvae captured per investigator hour of effort. We recorded the amount of investigator time necessary to complete each of the sampling efforts. We also assessed the material cost of each method and other ancillary costs when comparing method effectiveness, as available funding is often the limiting factor to the number and frequency of ponds that can be sampled.

4.2.3 General Larval Salamander Sampling

Although the 2005 sampling was designed primarily to compare method effectiveness, the results were also used as part of our general sampling to measure occupancy, larval capture rate, and detectability. In 2006 and 2007 we modified our sampling strategy and designed a sampling plan that included more ponds but less effort per pond (i.e., fewer trips) and sampling primarily with dipnets. The choice to conduct this sampling primarily with dipnets was based on results from the method comparison study. Dipnet sampling typically consisted of two or more netters entering a pond, selecting habitat (usually emergent grasses) that was considered of optimum quality and proceeding simultaneously with dipnetting in 5-min periods. At each interval between periods of netting, the number of larvae captured (if any) was recorded for each netter. This is similar to an approach reported by Alldredge et al. (2007) for bird counts.

In 2006 we sampled ponds at Fort Stewart from January through March when they dried, and we sampled ponds at Eglin AFB for a few days in February. In 2007 ponds at Fort Stewart never filled except for a few centimeters of water for a few days. Fortunately, we had arranged to sample at both St Marks NWR and Apalachicola NF for a week in late February. Although no sampling was planned for 2008, a slight delay in completion of the project allowed us to take advantage of a trip to Fort Stewart and sample for 1 day in February.

4.2.4 Larval Residency and Pond Hydroperiod

We evaluated possible hatching dates by matching observed sizes at capture with projected size at age based on modeled growth, which was initiated on dates of rain events that caused pond levels to rise and could have triggered hatching. Hatching of flatwoods salamander larvae on multiple dates within a season is not unusual (Palis 1995; Sekarek et al. 1996) and was considered in our analysis. We obtained daily rainfall data from two meteorological stations located equidistant (10 km) to the east and west of the single pond where larvae were found during this study and averaged the daily values. For modeling purposes we considered rain events in January and February that resulted in a daily total >20 mm or a running weekly total >25 mm as sufficient to result in a rise in pond levels.

Size at hatching information used in our model was based on total lengths reported by Anderson and Williamson (1976), which we converted to SVL based on the ratio of SVL:TL calculated for the larvae captured in this study (SVL=0.55·TL). They reported average lengths at hatching based on laboratory observations of 6.5 mm (SVL) on 25 November, 6.2 mm on 2 December, and 8.5 mm on 24 January after conversion. They also reported an average length of 7.0 mm (SVL) for newly hatched larvae captured in the field on 14 December. From these data we derived a relationship between date of hatching and mean SVL at hatching:

$$\text{Mean SVL}_{hatch} = 0.0446 \cdot \text{Julian day} + 7.658$$

We then subtracted and added 1 mm to include a measure of natural variation in size at hatching which became the origin of the minimum and maximum growth trajectories for a hatching date.

We also recognize that individual variation in growth exists, and, therefore, we used the range of growth rates calculated by Palis (1995) for two breeding sites in the Florida panhandle as minimum (1.78 mm/week; 0.254 mm/day) and maximum (2.54 mm/week; 0.363 mm/day) rates for the model. Although larval growth rates vary due to several factors, such as temperature, food availability, and densities of conspecifics and competitors, because we lacked any other growth data for this species, we assumed that larval growth at Fort Stewart was within the range reported by Palis (1995).

The growth trajectory modeled from a particular date was comprised of both minimum and maximum trajectories that created an envelope or cone of likely size at age. The maximum and minimum size-at-age lines are described by the following equations:

$$\text{Minimum trajectory SVL}_t \text{ (mm)} = (\text{SVL}_{hatch} - 1) + t * 0.254$$

$$\text{Maximum trajectory SVL}_t \text{ (mm)} = (\text{SVL}_{hatch} + 1) + t * 0.363$$

where t is time in days from hatching.

Lastly, we obtained survey results from the Fort Stewart Wildlife Branch Office of all the flatwoods salamander surveys at Fort Stewart since 1994 that included the dates and types (larval or adult) of surveys and the number and sizes of flatwoods salamanders found. These data were summarized for each of the 22 confirmed breeding sites and compared to area-wide rainfall as a surrogate for pond fullness.

4.2.5 Catch Rate – Abundance

Knowing the relative abundance of flatwoods salamanders from pond to pond and year to year is crucial for developing an effective conservation program. In addition, it is important for our analyses that we know the likelihood of collecting a larval salamander at different densities. Larval density can be measured by various mark-recapture methods or repeated removal sampling, but to do this at every pond with larval salamanders is not possible without nearly unlimited resources. Instead we chose to estimate larval density at a select few ponds and relate capture rate to density. In the future this will allow us to estimate larval density as a function of capture rate.

In the second and third year of the study we performed population estimates using the removal (or depletion) method (Moran 1951, Zippin 1958, Carle and Strub 1978) at several ponds so that we could derive a relationship between catch per unit effort for various sampling methods and salamander abundance. The removal method is often used when techniques like mark-recapture are impractical. It is useful for small populations or small areas and is quicker than mark-recapture since marking individuals is not necessary. The method requires three or more passes

(or sampling events) through a pond or portion of a pond. After each sampling, individuals are counted and removed or withheld from the area being sampled. Subsequent passes can be made immediately, so if specimens are temporarily maintained in holding tanks it would only be for a short time before they are returned to the pond. If only a portion of the pond is sampled then captured larvae can be immediately placed into portion of the pond not being sampled. The removal method assumes that changes in the population during the sampling period are only as a result of capture and that the probability of capture is equal for all individuals. We used the software CAPTURE to perform the maximum likelihood estimates of population size (White et al. 1982).

In 2006 we completed removal studies at one pond at Fort Stewart and one at Eglin AFB where flatwoods salamander breeding had been confirmed for that year. At the Fort Stewart pond we constructed an enclosure of approximately 10 x 40 m (400 sq m) using commercially available silt fencing. The enclosed area represented about 5% of the total pond area. At Eglin AFB the wetland being sampled had dried to a size that we were able to sample a small section of the wetland that was separate from other wetted areas and a physical enclosure was not necessary. The Eglin site was irregular in shape and estimated to be about 50-55 sq m.

Because we encountered low numbers of flatwoods salamanders at Fort Stewart and Eglin in 2006 and were unable to derive a capture rate: density relationship, we also estimated larval density at four ponds at St Marks NWR in 2007. The enclosures were constructed with silt fencing as at Fort Stewart; in addition, 20-cm long staples made from coat hangers were used to secure the bottom of the silt fence to the pond bottom between wood stakes (Figures 6 and 7). Enclosure sizes were either 6 x 12 m (72 sq m) or 9 x 9 m (81 sq m). Because the ponds were completely full, the enclosed areas were < 5% of the total pond areas.

At least three passes were made through each enclosure with 3-4 netters. The length of time for each pass was consistent within a pond enclosure, but varied among ponds depending on the size of the enclosure and the number of netters. We recorded the number of larvae captured by each netter for each pass. Captured larvae were placed in containers outside of the enclosures until all passes were completed.

4.3 RESULTS AND ACCOMPLISHMENTS

4.3.1 Method Comparison

For the method comparison study we sampled eight ponds at Fort Stewart in 2005 and three at St Marks in 2007 with both dipnets and traps. Our method comparison in 2005 included 1,744 minutes of dipnetting coincident with 1,794 total trapnights (838 metal traps, 840 plastic traps, and 116 box traps) at eight ponds. At Fort Stewart we recorded data on all amphibians, crayfish, and fish captured (Figure 8). Amphibians (primarily tadpoles) were caught most frequently by dipnetting followed by the two minnow traps at a similar rate. Nearly all flatwoods salamanders were captured by dipnetting. Dipnetting was the most effective method for capturing crayfish, though the minnow traps were also effective. Fish were more susceptible to capture by the metal

Fig. 6. Dipnet sampling during removal study at St Marks pond.

Fig. 7. Pond enclosure being dismantled after completion of removal study at St Marks pond.

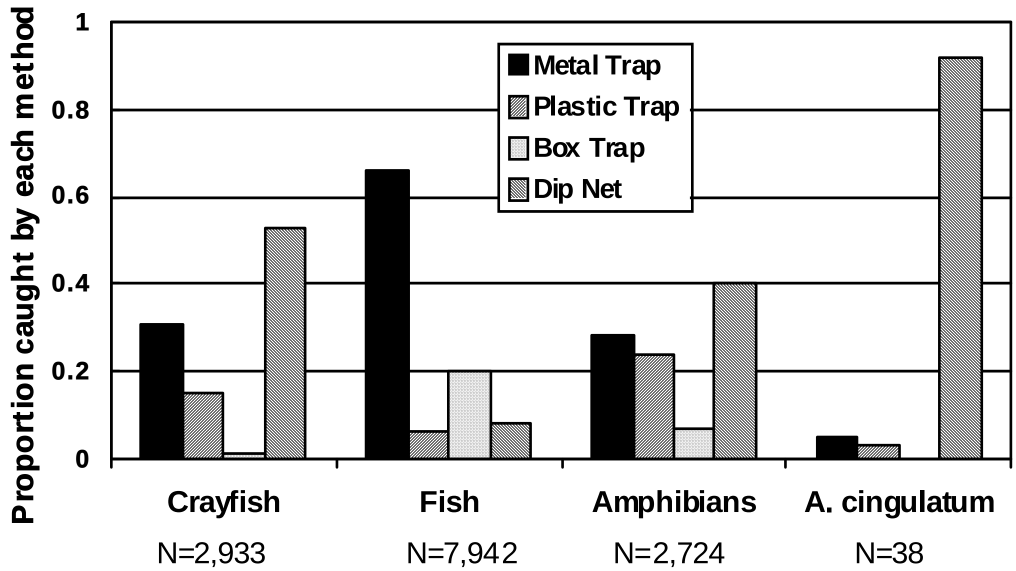


Fig. 8. Proportion of organisms caught by four collection methods for four taxa during method comparison study at Fort Stewart in 2005.

minnow traps than other methods. Plastic minnow traps of nearly the same dimensions caught 10 times fewer fish than metal traps.

At Fort Stewart, dipnetting produced significantly more larvae than any of the traps we tested (Table 2). Because the typical unit of effort for trapping is number of nights set and the unit of effort for dipnetting is minutes netted, we chose to standardize effort for comparison based on actual hands-on investigator time needed to use each method. After standardization the amount of effort at each site within a pond was similar for all methods. Our typical dipnetting effort per site was 5 minutes of netting by two people for a total of 10 minutes. It also took about 10 minutes for two people to set and check eight minnow traps (plastic or metal), which was the number normally placed at a site overnight. A single box trap took about 5 minutes to set and check because it often captured more individual organisms and took longer to empty because of its design. When standardized to per minute of investigator effort, we found that dipnetting was roughly 5-10 times more effective than the traps. Since flatwoods salamander larvae were only captured in one pond, we limited our analysis to only those dates when we were certain larvae were in the pond. Based on data from 20-21 April and 2-4 May (Table 3), metal traps captured two larvae in the equivalent of 146 minutes (0.8/hour), plastic traps captured one larva in the equivalent of 145 minutes (0.4/hour), and dipnetting captured 27 larvae in 431 minutes (3.8/hour). The data also show the decline in dipnetting effectiveness that occurred when numbers in the pond declined as larvae metamorphed and left the pond late in the season.

At St Marks in 2007, the difference in effectiveness between dipnets and traps was not as pronounced as at Fort Stewart (Tables 2 and 3). On average, dipnetting produced 2.5 and 3 times more larvae than metal and plastic traps, respectively. In addition, the average dipnet capture rate

Table 2. Trapping and dipnetting effort in ponds with flatwoods salamander larvae present at Fort Stewart and St Marks (number of larvae captured in brackets)

Date or Pond ID	Metal traps (trapnights)	Plastic traps (trapnights)	Box traps (trapnights)	Dipnet (minutes)
2005 FORT STEWART (Alpha pond)				
Mar 1-3	44	44	6	122
Mar 21-23	48	48	6	60
Apr 21-22	47	48 [1]	--	220 [14]
May 3-5	72 [2]	72	7	211 [13]
May 11-13	--	--	--	244 [5]
May 17	--	--	--	89 [2]
May 23-24	--	--	--	85 [1]
May 31-Jun 2	---	--	--	165
TOTAL	**211 [2]**	**212 [1]**	**19**	**1,196 [35]**
2007 ST MARKS NWR (Feb. 20-22,2007)				
SMNWR0111	20 [3]	20 [10]	--	65 [32]
WAKDC0009	20 [2]	20 [2]	--	60 [28]
SMNWR0110	24 [10]	24 [4]	--	70 [45]
TOTAL	**64 [15]**	**64 [16]**	--	**195 [105]**

Table 3. Standardized catch per effort (#/min) for different collection methods during weeks when flatwoods salamanders were detected at Fort Stewart and St Marks

2005 FORT STEWART (Alpha pond)				
Sampling dates	**Metal traps**	**Plastic traps**	**Box traps**	**Dipnet**
Apr 21-22	0	0.008	--	0.064
May 3-5	0.011	0	0	0.062
May 11-13	--	--	--	0.020
May 17	--	--	--	0.022
May 23-24	--	--	--	0.012
AVERAGE	**.0067**	**0.0033**	**0**	**0.041**

2007 ST MARKS NWR			
Pond ID	**Metal traps**	**Plastic traps**	**Dipnet**
SMNWR0111	0.12	0.40	0.49
WAKDC0009	0.08	0.08	0.47
SMNWR0110	0.33	0.13	0.64
AVERAGE	**0.18**	**0.20**	**0.54**

at St Marks was nearly 10 times the best daily rate at the Fort Stewart Alpha pond, which was no doubt a function of greater larval abundance in the St Marks ponds.

In addition to analyzing the capture efficiency of the different collection methods, we were also interested in their economic efficiency. As part of our analysis of the different methods, we also took into account the material costs, time to transport equipment to the ponds, and time to move from site to site within a pond. Costs and durability were compared for the four capture methods used in the comparative study (Table 4). With these additional considerations we conclude that dipnetting is the most cost efficient and preferred method for capturing flatwoods salamanders.

Table 4. Comparison of different sampling methods used to capture flatwoods salamander larvae

Method	Material Cost	Durability	Cost Effectiveness
Dipnetting (monorail hoop net design with 3/16 inch nylon mesh)	$16.10/net	Dipnets will generally last one season or less. Rips in netting will occur when snagged on branches and other debris. Handles break and bend due to vigorous action required to be effective.	- highest capture rate for *A. cingulatum* - requires only one surveyor - inexpensive, lightweight, fairly durable
Plastic Funnel Trap (cylindrical, two halves hinged together with clasps for closure, two cone-shaped funnel entrances - 3/16 inch mesh)	$6.90/trap	Plastic traps are durable and will likely last two to three field seasons. Locking of clasps may become difficult with wear.	- low capture rate for *A. cingulatum* - may require more than one surveyor in order to transport in volume - inexpensive, fairly lightweight, durable
Galvanized Metal Funnel Trap (cylindrical, two halves held together with clips, two cone-shaped funnel entrances – 1/8 inch mesh)	$21.00/trap	Galvanized metal traps are durable and could last at least five field seasons. Misplacing clips is greatest concern.	- low capture rate for *A. cingulatum* - may require more than one surveyor in order to transport in volume - relatively expensive, fairly heavy, but durable
Box Trap (wood frame box, two full length funnel entrances - 1/8 inch wire mesh)	$89.50/trap	Large square minnow traps are fairly durable; however, may need repair during or after field season. Mesh may develop holes and wooden frame is susceptible to damage with rough treatment.	- no *A. cingulatum* captures - one person can only carry two traps at a time - expensive, fairly durable

4.3.2 Flatwoods Salamander Detections

Fort Stewart

In mid February 2005 when we first visited six known ponds at Fort Stewart for sampling they were dry except for a few small (2 x 2 m) shallow pools. By late February these ponds had filled to a depth sufficient for sampling (approx. 30 cm maximum). No flatwoods salamander larvae were captured during February and March after sampling an average of 7 days each at six ponds with dipnets and traps and a reduced level of sampling at two other ponds. Sampling effort was reduced in April due to concerns that earlier dry conditions had resulted in reproductive failure, but on 21 April we captured a larva at a known breeding pond (henceforth referred to as Alpha pond) in a trap followed by 14 more individuals by dipnetting (Figure 9). From 21 April to 23 May we captured a total of 38 larvae from Alpha pond during seven visits (see Table 2). No other larvae were found in Alpha pond during sampling from 31 May – 3 June, and none were found in follow-up surveys of other ponds during late April and May.

Fig. 9. Larval flatwoods salamander (approx 70 mm total length) collected from a Fort Stewart pond in April 2005.

All 15 larvae captured on the first day of discovery (21 April) were found throughout the central portion of the main pond; peripheral areas were not sampled (Figure 10). Nearly two weeks later, we were only able to find one larvae in this portion of the pond after which we discovered several fingers or trenches of water along the outer edge of the pond. Upon investigation we discovered several more larvae (15) in these areas over the next few days. These trenches were created by logging operations several years ago and were completely re-vegetated (Figure 11). Although some of those captured in the second round may have been recaptures from the first, we strongly doubt this based on the observed lengths and the expected growth rates (see Section 4.3.3). Flatwoods salamanders typically metamorphose at around 40-45 mm snout-vent length.

Assuming a growth rate of around 2 mm per week, many of those captured were within a couple weeks of leaving the pond. We also compared individual markings from photographs taken of both groups and found no evidence that would suggest some individuals were recaptured.

During sampling in the third week of May, we found no larvae in the central pond, but another five in the logging skidder tracks. The capture rate continued to decrease through the rest of May. From 11 May through 23 May we only sampled using dipnets since we had completed our planned comparison study and because dipnetting appeared to be a superior method.

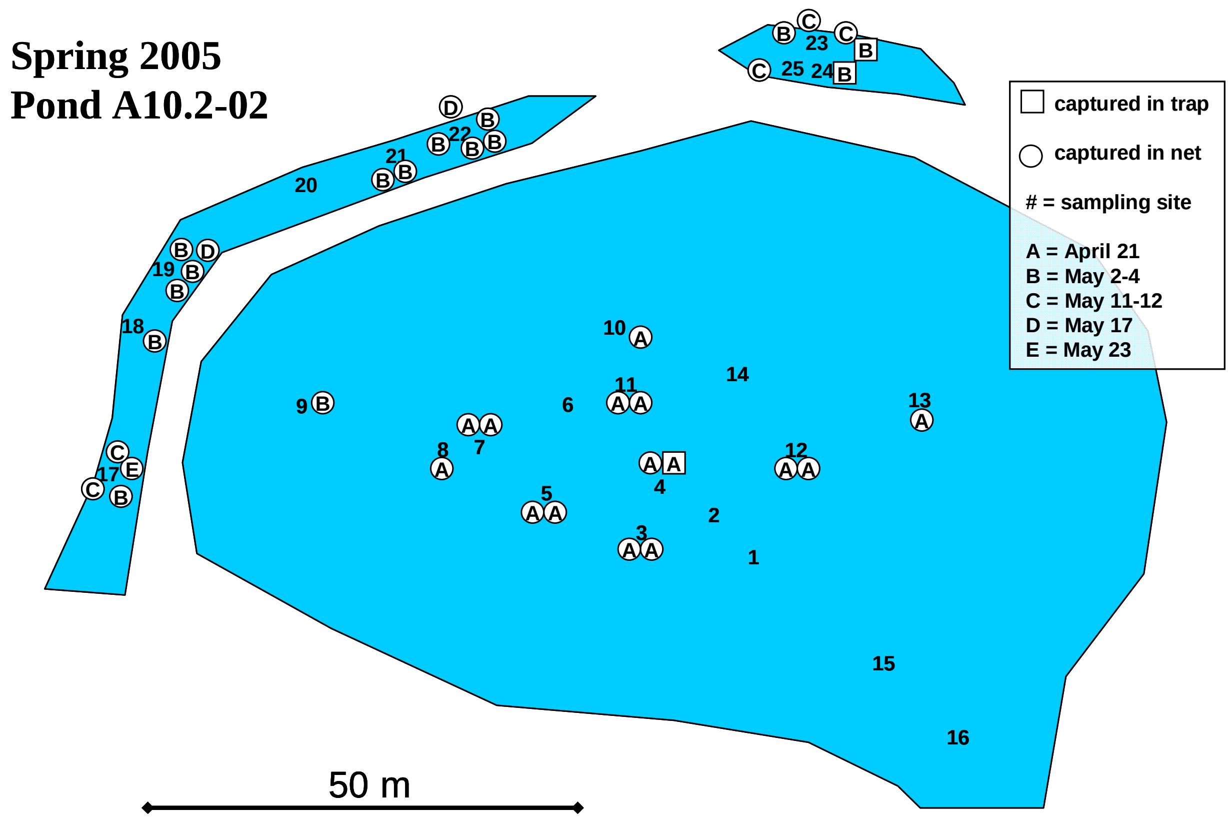


Fig. 10. Specific capture locations for 38 flatwoods salamanders captured in Alpha pond in 2005. Shape of symbol (circle or square) indicates method of capture. Letters A through E provide the key for date of capture.

In 2006 the ponds at Fort Stewart had filled when sampling began in the middle of January. We sampled a total of 60 ponds (21 known breeding ponds and 39 potential breeding ponds) which ranged in size from about 0.14 to 7.5 ha. Most ponds (46) were sampled only once, 13 were sampled two to three times, and the single pond where flatwoods salamander larvae were found during this study was sampled on eight occasions. We captured two flatwoods salamander larvae at Alpha pond during the first visit on 18 January. On four subsequent visits (27 January, 7-8 February, 8 March, and 4 April), 27 additional larvae were captured by dipnetting. Ponds dried during March, and larvae captured on 4 April were dipnetted from among the few remaining

Fig. 11. Parallel logging skidder tracks along the periphery of a Fort Stewart pond.

shallow pools located within the Alpha pond basin. Salamander sampling continued until most of the ponds dried in April. Unlike in 2005 when larvae were found throughout the pond, nearly all the larvae captured in 2006 were from one end of the unnatural trenches (Figure 12). We found no larvae in the other 59 ponds sampled in 2006, including one that is within 100 m of Alpha pond.

In 2007, the depressions were virtually dry throughout the winter and spring and we were unable to sample.

Because the project was originally scheduled to be completed by the end of 2007, we had not planned to sample in 2008. However, a trip in February 2008 to brief Fort Stewart biologists on the project findings provided an opportunity to sample five ponds, including the only recently confirmed breeding pond. One larvae was found within the first minute of dipnetting and after a total of 105 minutes of dipnetting by 3 netters, we captured nine larvae. Two larvae were found in the skidder trenches at the southeast corner of the wetland and the rest at the opposite end of the pond.

Larval sizes captured in the Alpha pond during the period of the study are summarized in Figure 13. Detailed information on size, date of capture, and method of capture is included in Appendix A.2. A list of all ponds sampled for larvae at Ft Stewart and elsewhere during the course of the study and the years each was sampled is included in Appendix A.3.

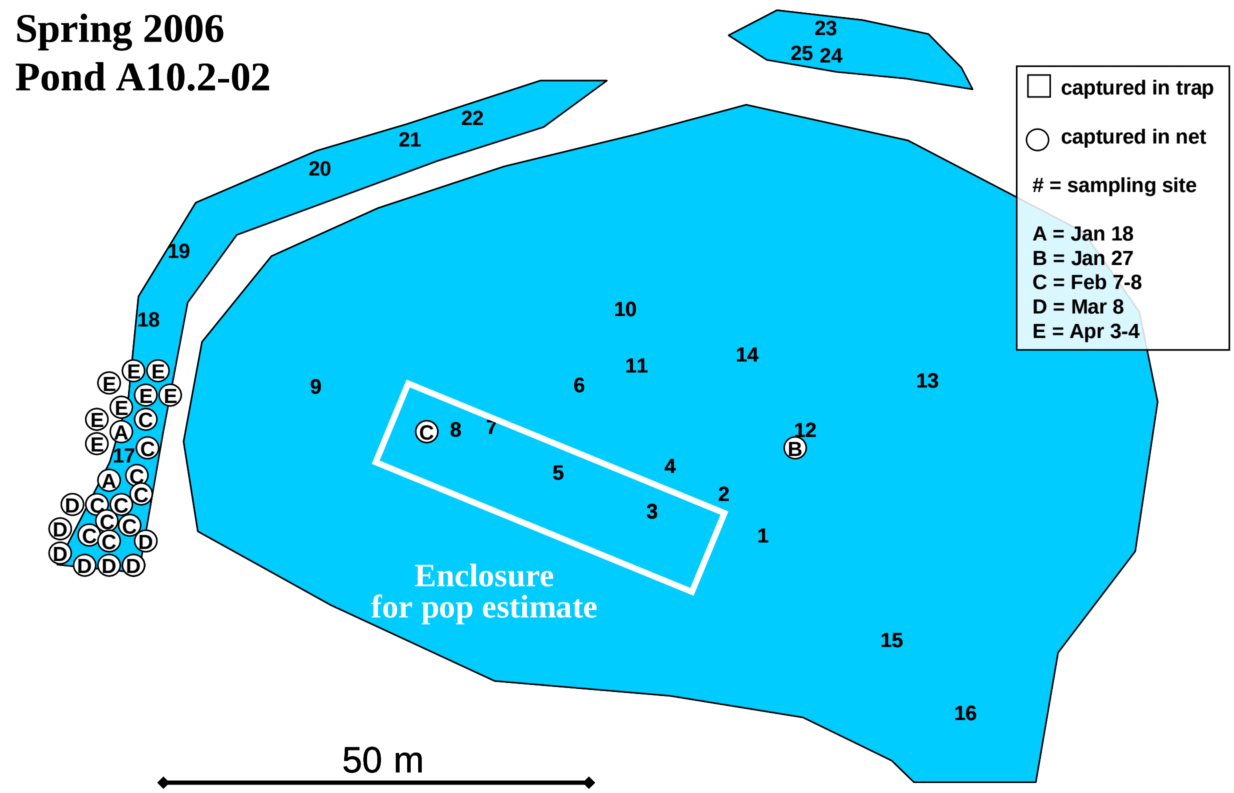


Fig. 12. Capture locations for 29 flatwoods salamander larvae captured in Alpha pond in 2006. Symbol shape (circle or square) indicates method of capture. Letters A through E provide the key for date of capture.

Eglin AFB

We extended our sampling in 2006 to include a week at Eglin Air Force Base (Florida) in case conditions (i.e., precipitation and pond level) were not adequate at Fort Stewart. Even though precipitation was adequate at Fort Stewart, we still completed the trip to Eglin since we only found salamanders at a single pond at Fort Stewart. As it turned out, conditions at Eglin were drier than normal, many of the known ponds dried early or never filled, and we found flatwoods salamanders at only one Eglin pond. Of the Eglin ponds that we were able to sample, this was the only pond where flatwoods salamanders had been found by other investigators earlier in the season (about two months prior to our sampling). We collected flatwoods salamanders within the first five minutes of dipnetting from the last remaining pools of a drying pond. These pools were largely found in what appeared to be a fire break that had been dug through the pond years earlier.

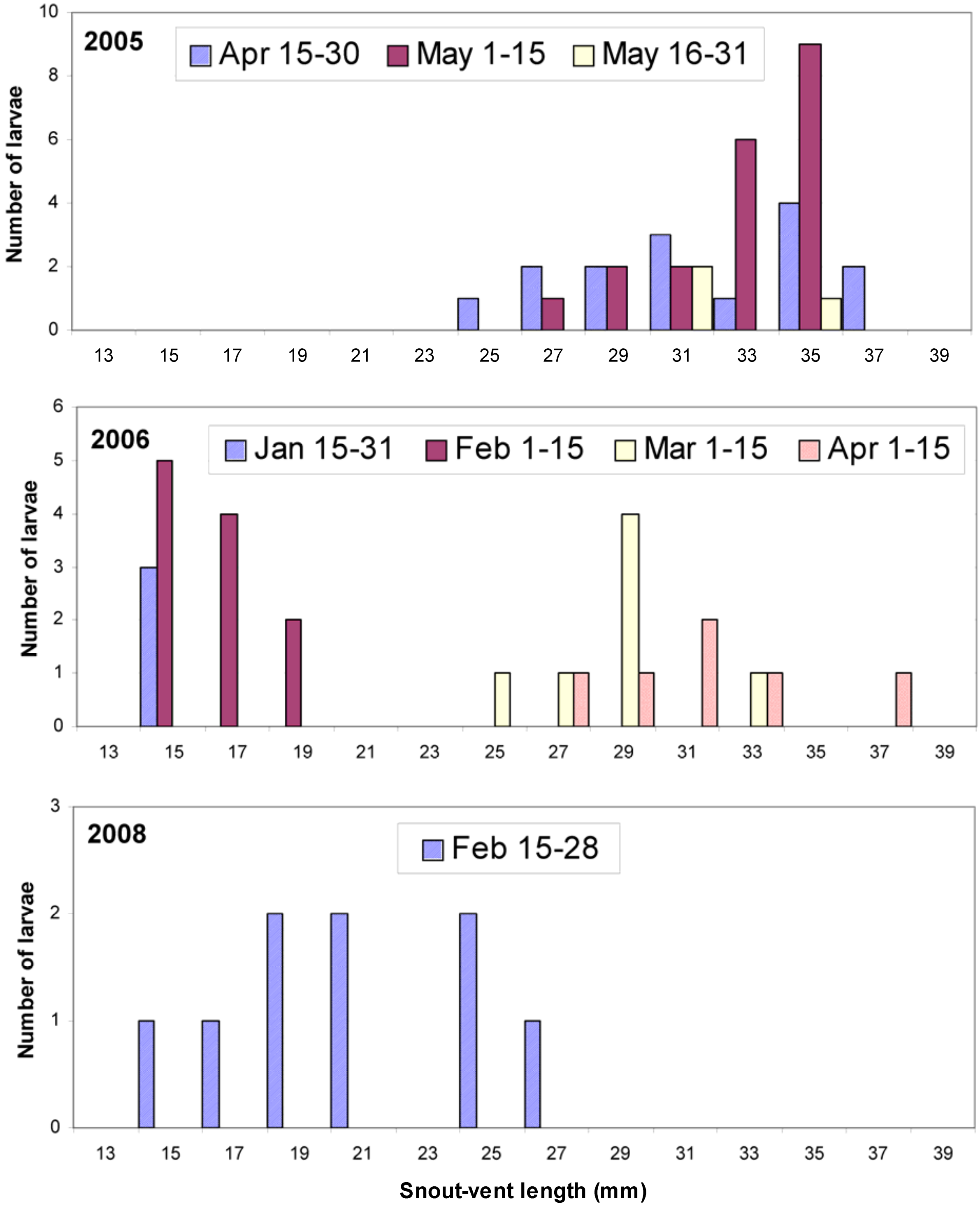


Fig. 13. Length frequencies of flatwoods salamander larvae captured at Fort Stewart 2005-2008.

St Marks National Wildlife Refuge

In 2007 we sampled by dipnetting seven ponds for two days in late February at SMNWR. Three of these ponds were also sampled with traps. We were assisted on this trip by Kevin Enge, state herpetologist for the Florida Fish and Wildlife Conservation Commission, and John Palis, flatwoods salamander expert who has several publications on the species. Over 350 flatwoods salamander larvae were collected and released at SMNWR from 5 previously confirmed ponds and 2 previously unconfirmed ponds (that were not distinguishable as separate ponds due to the high water levels). As at Fort Stewart we recorded numbers captured for each netter at 5-min intervals. Larvae were held briefly to be counted and measured before return to the ponds (Figure 14). Larval sizes are summarized in Figure 15.

Fig. 14. Flatwoods salamander larvae captured at St Marks NWR, Florida in February 2007.

Apalachicola National Forest

In 2007 we sampled by dipnetting five ponds on February 22 at ANF. We collected and released 49 larvae from 3 previously confirmed ponds. On average, larvae captured at ANF were smaller than those collected during the same week at SMNWR (Figure 15).

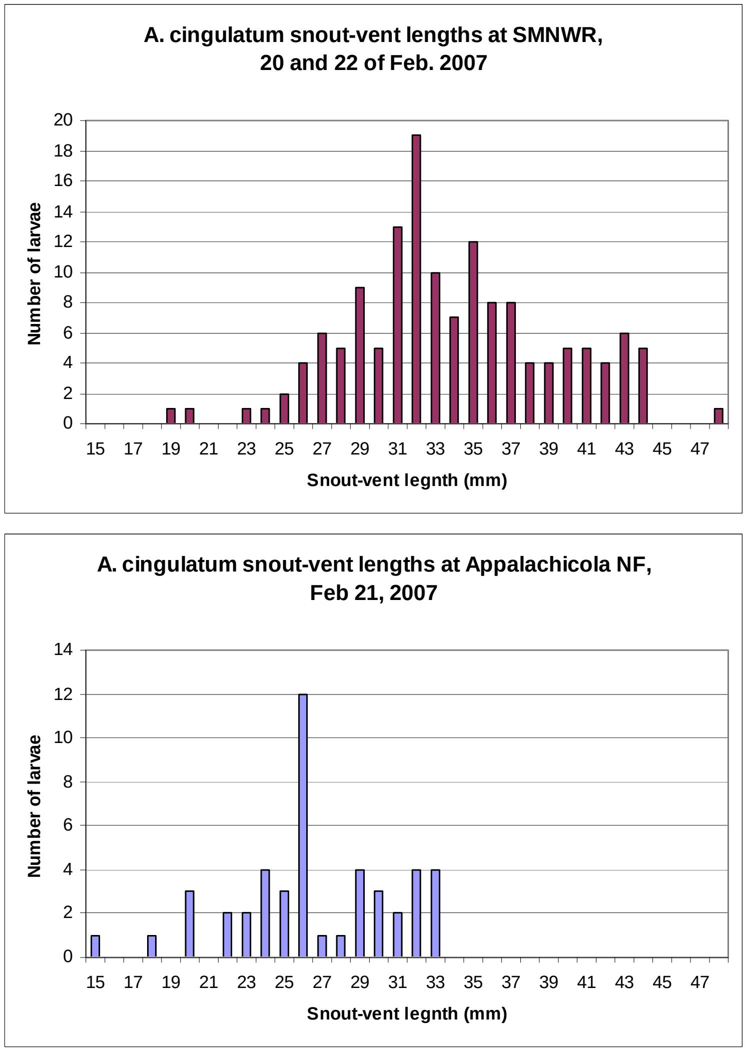


Fig. 15. Length frequencies of flatwoods salamander larvae captured at St Marks and Apalachicola in late February 2007.

4.3.3 Larval Residency and Pond Hydroperiod

Our analysis of the Fort Stewart rainfall data revealed that in 2005 three dates from 1 January to 28 February met the criteria we established for what was necessary to raise pond levels (14 January, 3 and 28 February) and in 2006 four dates (2 and 24 January, 3 and 26 February). We produced size-at-age envelopes (i.e., minimum and maximum growth trajectories) for each of these dates and evaluated how well the size envelopes encompassed the observed larval sizes. Figure 16 shows an envelope from the most likely hatching date defined as the first day with daily rainfall of >25 mm and another from the remaining hatching dates that encompassed the greatest number of the remaining larval sizes.

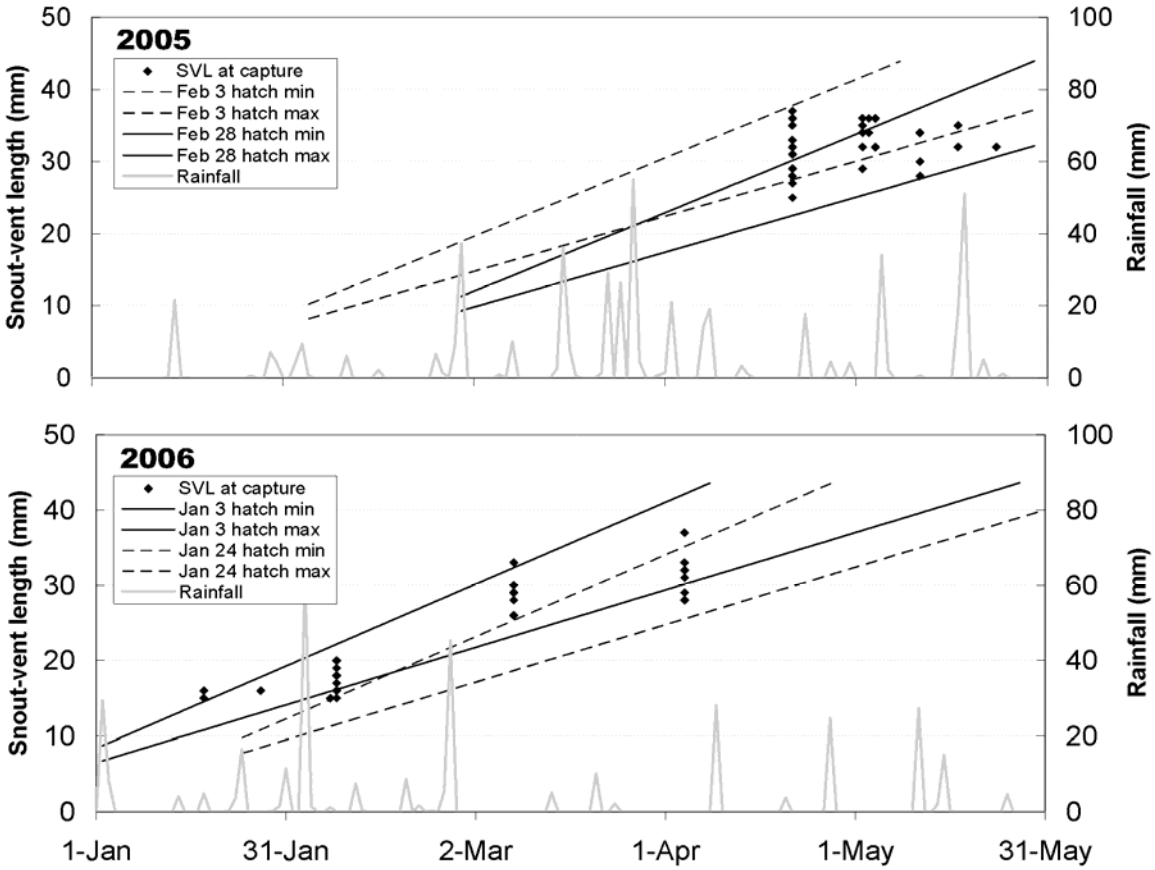


Fig. 16. Snout-vent length (mm) for larval flatwoods salamanders by capture date from Fort Stewart Alpha pond. Envelopes of modeled size-at-age for two possible hatching dates for 2005 and 2006 are shown as solid lines (most likely date of hatching) and dashed lines (second most likely). Daily rainfall (mm) is indicated by the gray line.

Biologists at Fort Stewart have maintained comprehensive records of flatwoods salamander sampling effort and captures at 22 confirmed breeding sites over the past 13 years (Figure 17). Prior to the 2 years of sampling reported here, nearly all of the sampling occurred in February to early April. Of 86 sampling trips to known ponds from 1994 to 2004 only three ponds were sampled in January and none after 13 April. These records show a decline since 1994 in the proportion of confirmed ponds surveyed in a given year that contained flatwoods salamanders. Nearly every previously confirmed pond that was sampled in 1994 (18 of 19 sampled) produced larvae. In the late 1990s, about half of the confirmed ponds that were sampled each year had larvae present. A protracted drought (1999-2002) experienced throughout the Coastal Plain of Georgia and South Carolina resulted in four consecutive years of potentially complete reproductive failure at most breeding sites. Although some pond basins on Fort Stewart were partially inundated in 1999, pond hydroperiods were of insufficient duration to allow larval development through metamorphosis (D. Stevenson, unpublished data). In the past 4 years, we only found larvae in 1 of 21 known ponds sampled even though survey effort (based on pond visits) exceeded most previous years.

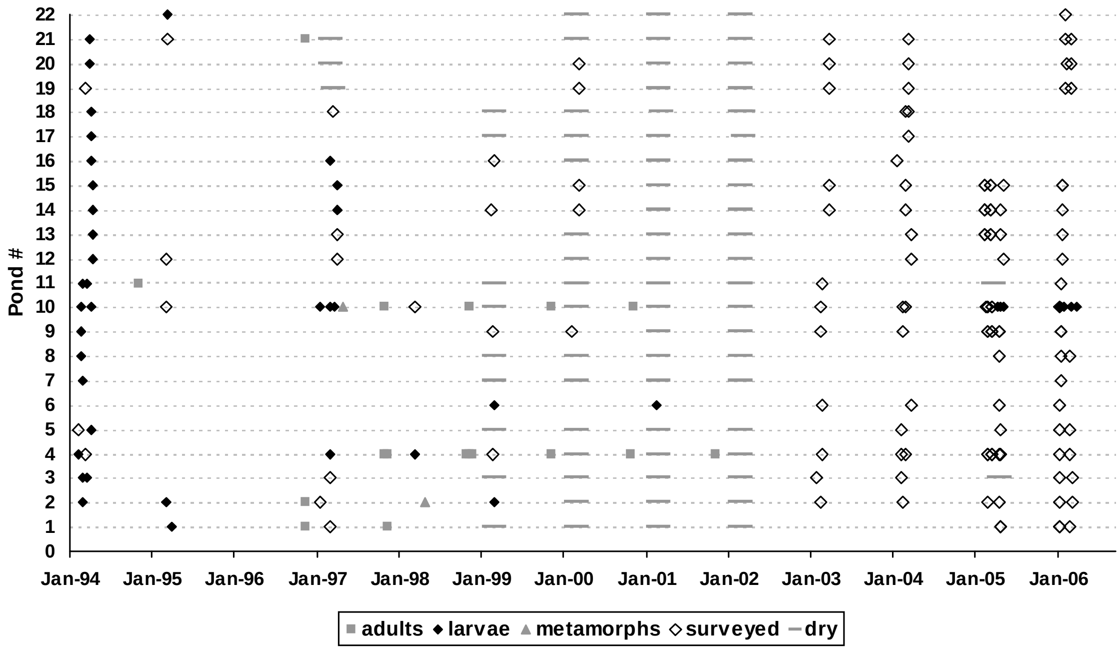


Fig. 17. Compiled survey history for flatwoods salamanders on Fort Stewart from 1994-2006 showing incidences of unsuccessful and successful surveys for larval salamanders (open and filled diamonds), observation of adults or metamorphs outside of the ponds (squares), and periods when pond were dry and could not be sampled (lines).

The historical records also contain size information for many of the collections. A summary of observed sizes by date since 1994 helps explain the likely periods of pond residency (Figure 18).

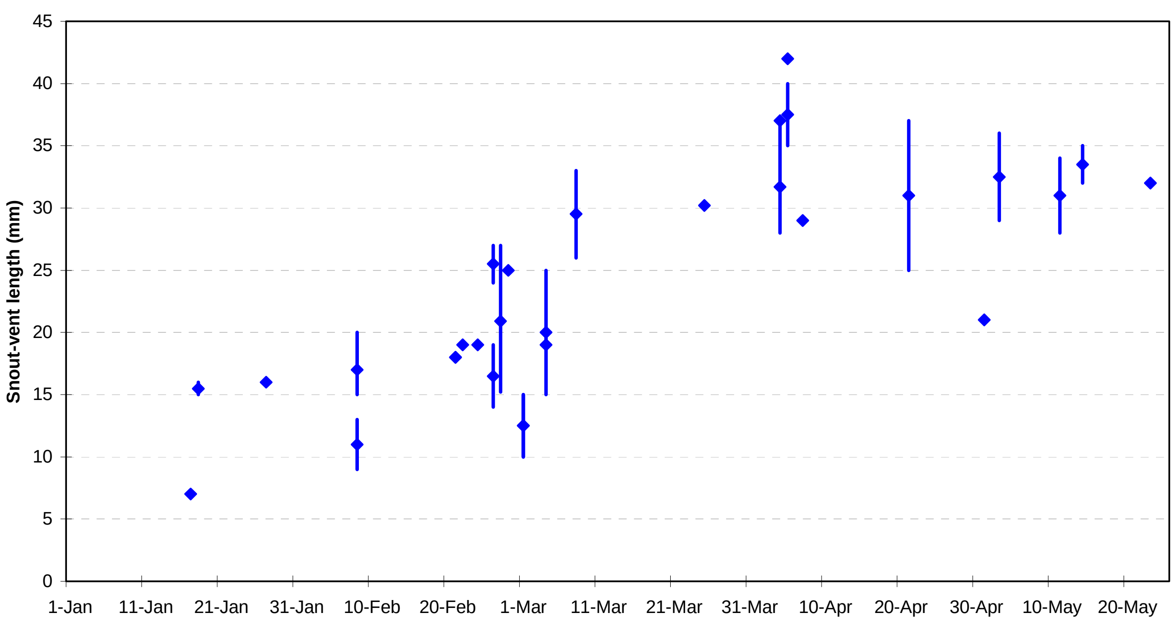


Fig. 18. Mean and range of snout-vent lengths for flatwoods salamander larvae collected at Fort Stewart from 1994-2008.

For example, larvae within a few millimeters of the likely size at hatch are observed throughout January and February, suggesting that hatching may occur at least as late as the end of February. The maximum observed sizes which would occur just before metamorphosis and migration from the ponds occurred as early as early April and until the end of May.

The historical data were also useful for investigating the relationship between rainfall and sampling success. Figure 19 shows the relationship between survey dates (both successful and unsuccessful) since 1994 and rainfall data during the period of likely larval residency for those years. January-February rain was typically low in years when surveys were unsuccessful (i.e., 1996, 2000, 2001, 2002, 2003, and 2004). In 2004, similar to 2005, rainfall was slight until the end of February, and surveys were discontinued at the end of March. Had surveys continued through April, larvae may have been found in 2004 as they were in 2005.

4.3.4 Catch Rate – Abundance

We completed population estimates by the removal method using enclosures in four ponds at St Marks (Table 5). Estimated larval densities varied from 60 to 108 individuals per 100 m^2.

We found a significant relationship between catch rate and salamander density. Although catch rate is a function of density, Figure 20 was plotted with density on the dependent axis, because we believe it is useful for investigators to be able to estimate larval density based on catch rate. At the completion of the study we felt that each pass through an enclosure was probably at a greater effort per square area than we would normally do and therefore believe that the catch rates are probably slightly higher than what we would observe under normal sampling at the observed density.

Catch efficiency (i.e., the number of larvae captured per pass divided by the total number in the enclosure) averaged 0.40 and ranged from 0.22 to 0.64, but there was no relationship with density (Figure 21). That is, catch efficiency did not decline as abundance dropped with successive passes.

4.4 DISCUSSION

4.4.1 Methods Comparison

Our study demonstrates that dipnetting is more effective for surveying flatwoods salamander larvae than passive traps. Although checking a single trap for larvae can be done in less than a minute, the time it takes to transport the traps to the pond, distribute them throughout the pond, and locate the proper depth for deployment accumulates to significant time expenditure for minimal return. Traps were more effective at St Marks, where densities were very high, than at Fort Stewart.

It is possible that variation in dipnetting technique among netters can produce different results with regard to capture success, and we tried to standardize the technique that we use to minimize investigator variation. Our dipnetting technique was patterned after one used by experienced

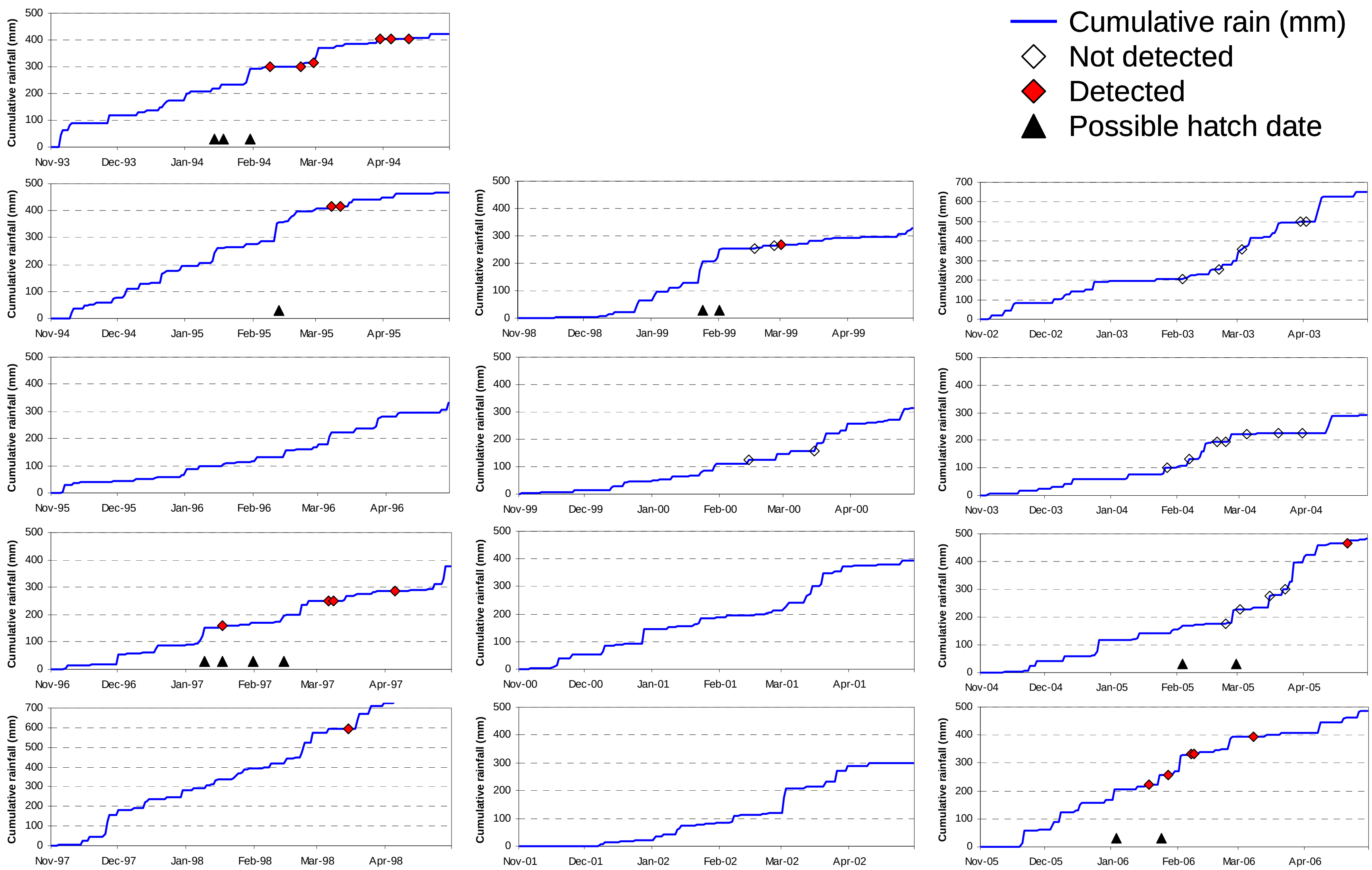


Fig. 19. Dates for 1994-2005 of unsuccessful and successful larval surveys at Fort Stewart superimposed on cumulative rainfall since November of the previous year.

Table 5. Results of removal method population estimates in enclosures at four St Marks ponds

Pass #	Min. per pass	Larvae captured	Cumulative number captured	Catch rate (#/min)	Est. population size	Enclosure area (m^2)	Density (# per 100 m^2)	Catch efficiency
Pond SMNWR0111 – Estimated population = 60 (95% CI = 56 to 78)								
1	60	30	30	0.50	60	83.6	71.8	0.50
2	60	17	47	0.28	30	83.6	35.9	0.57
3	60	7	54	0.12	13	83.6	15.5	0.54
Pond WAKDC0009 – Estimated population = 80 (95% CI = 65 to 134)								
1	30	20	20	0.67	80	74.3	107.6	0.25
2	30	17	37	0.57	60	74.3	80.7	0.28
3	30	16	53	0.53	43	74.3	57.9	0.37
4	30	6	59	0.20	27	74.3	36.3	0.22
Pond SMNWR0110 – Estimated population = 50 (95% CI = 46 to 68)								
1	30	19	19	0.63	50	83.6	59.8	0.38
2	30	7	26	0.23	31	83.6	37.1	0.23
3	30	9	35	0.30	24	83.6	28.7	0.38
4	30	5	40	0.17	15	83.6	17.9	0.33
5	30	4	44	0.13	10	83.6	12.0	0.40
Pond SMNWR0109 – Estimated population = 47 (95% CI = 45 to 62)								
1	30	19	19	0.63	47	74.3	63.2	0.40
2	30	18	37	0.60	28	74.3	37.7	0.64
3	30	6	43	0.20	10	74.3	13.5	0.60
4	30	1	44	0.03	4	74.3	5.4	0.25

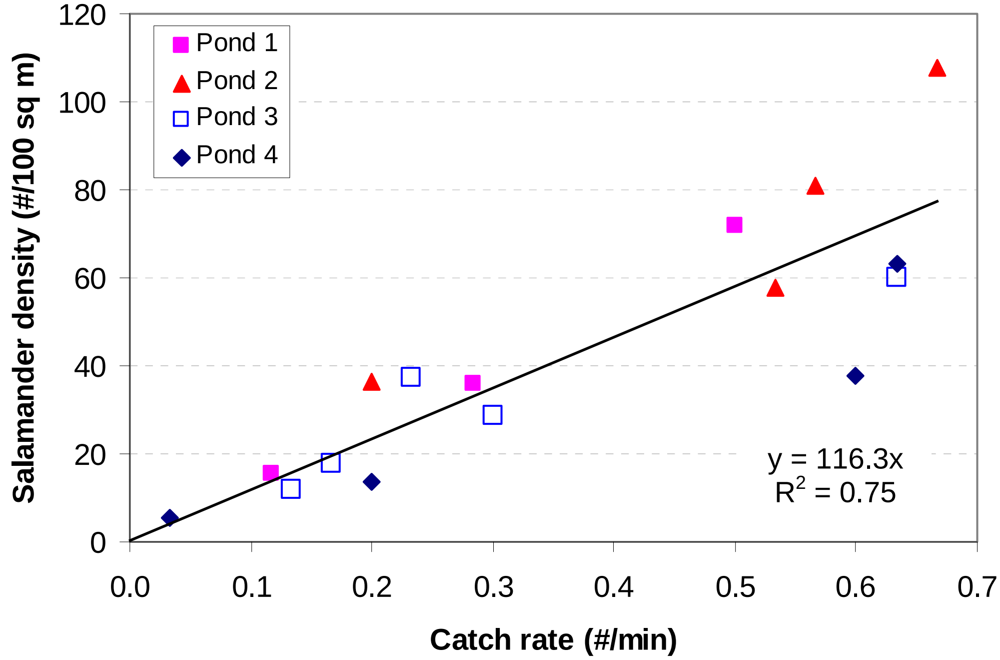


Fig. 20. Positive relationship between team catch rate and larval density determined during each pass of four removal studies.

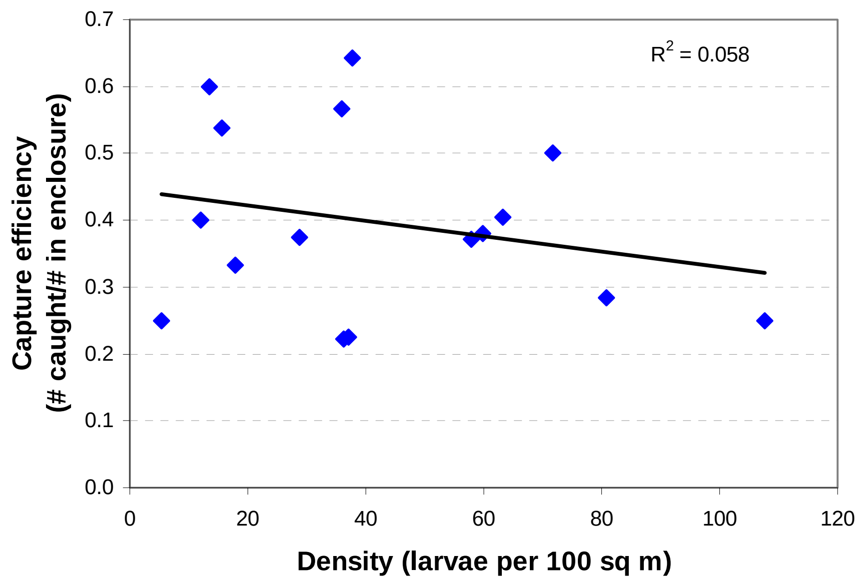


Fig. 21. Lack of relationship between larval density and capture efficiency during removal study. Each point represents data from one of 16 passes during the four enclosure studies (values from columns 8 and 9 in Table 5).

flatwoods salamander biologists (Palis 1996). We recorded capture data by investigator and, although we did not perform a rigorous statistical analysis, we did not notice any difference in capture rate among dipnetters that could result from differences in technique or experience.

Palis (1996) suggests that a minimum sweep area of 16 sq m may be needed to find flatwoods salamander larvae when abundance is low. The lowest catch rate we observed was 1 larva in 85 min of dipnetting. It take us about 5 min to cover 16 sq m, so it seems that the minimum sweep area at low densities could be as much as 17 (85/5) times higher.

Night-time sampling with this method may be more effective since flatwoods salamander larvae are nocturnally active, but can be more difficult because of the logistics of working in the dark or with artificial light. We were not able to test the effectiveness of night sampling because of complications with working at night on an active military base. If one does use traps for sampling, we suggest that the entrances be modified such that they are small enough to allow salamander larvae to enter but not large crayfish that might prey on captive larvae.

One objective of the methods comparison task was to compare the relative costs of deploying various sampling techniques. Because of its experimental nature, the sampling performed during this study was not designed as a sampling plan that one might adopt for long-term monitoring or for occupancy determination. However, we used what we learned during our sampling to generate two potential sampling scenarios, one using just dipnetting and the other a combination of dipnetting and trapping, to compare estimates of the material, effort, and cost (Table 6). By adding trapping and keeping the time spent dipnetting constant, fewer ponds (or pond-days) could be sampled using the second alternative. Because many more traps are needed than dipnets when trapping is added, the material cost of the second alternative is greater. Dipnetting is not only less expensive an alternative, but also, as noted earlier, much more effective at capturing flatwoods salamander larvae. We kept the total amount of effort (8 weeks) the same for both alternatives so that labor cost is the same for both. The dipnetting only alternative produced more total pond visits than the combination alternative, but reducing the amount of time dipnetting each pond would allow more ponds to be visited under the combination alternative and equalize the number of pond visits. There are numerous combinations of various levels of effort and sampling techniques that could be evaluated, but because the primary expense is labor, the cost of any sampling scenario is largely driven by how much investigator effort can be invested.

4.4.2 Fort Stewart Detection Trends

Flatwoods salamander larvae captured in 2005 were the first captured at Fort Stewart from a natural wetland since 1999; larvae were found in a former borrow pit in 2001 (Figure 17). Since 2002, repeated surveys at known breeding sites elsewhere in Georgia and in South Carolina have found larvae at just one site in South Carolina (a single larva found in 2003 at a site on Francis Marion National Forest; Steve Bennett, South Carolina Department of Natural Resources, personal communication) and at one site in Georgia (single larva found in each of 2001 and 2003 from adjacent wetlands on Townsend Bombing Range; John Jensen, Georgia Department of Natural Resources, and Win Seyle, U.S. Army Corps of Engineers, personal communication). Biologists have found flatwoods salamanders on a more frequent basis at many sites in Florida during this period (Kevin Enge, Florida Fish and Wildlife Conservation Commission, personal

Table 6. Material, effort, and cost comparison for two single-season sampling strategies. Labor costs assume an hourly rate of $25 for field technicians. Trap cost is based on the average cost for the two minnow trap types.

	Dipnetting only	Dipnetting and trapping
Ponds per day	5	3
Hours/pond/day	1	1.5
Days netting per week	4	4
Nights trapping per week	0	4
Dipnets for season (cost)	12 ($20 ea. = $240)	8 ($20 ea. = $160)
Traps for season (cost)	0	90 (30/pond) ($14 ea. = $1,260)
Weeks of sampling	8	8
Crew size (labor cost)	2 (~$400/day)	2 (~$400/day)
Total pond visits	160	96
Total cost per season	$16,240)	$17,420

communication) including high numbers observed at St Marks National Wildlife Refuge in late February 2007 during this study.

Lastly, we do not know whether the high level of occurrence reported in 1994 at Fort Stewart was the result of ideal hydrologic conditions, a peak in a cyclic pattern of natural population fluctuation, a result of greater survey effort, or a combination of these and other environmental factors. Palis et al. (2006) observed a decline in the number of breeding adult flatwoods salamanders over 4 consecutive years at a breeding pond in Florida and attributed this decline to adult attrition, lack of juvenile recruitment, and lack of rain or abnormally low rain during the period of breeding migrations. Similarly, we suspect that adult attrition and lack of juvenile recruitment due to the drought are responsible for the putative decline of flatwoods salamanders on Fort Stewart and elsewhere in Georgia and South Carolina. If the conservation and preservation of this and other rare amphibian species is to be successful, biologists must identify and utilize which survey methods are most effective and should maximize the likelihood of detection through a better understanding of the relationship between pond residency and various environmental factors.

4.4.3 Larval Residency and Pond Hydroperiod

We suggest that the most opportune time of year to sample flatwoods salamander larvae is during the second and third months after a pond fills to at least half full. In Florida metamorphosis of flatwoods salamander larvae is usually complete by April (Sekarek et al. 1996), but is likely later on average at more northern latitudes. In the Fort Stewart region, April-May is a period of reduced rainfall and pronounced evapotranspiration; thus, pond water levels recede rapidly during this time (Palis 1997). Bishop et al. (2006) recommended that surveys for flatwoods salamander larvae be conducted primarily from February to early April, but depending on various weather-related factors sampling in other months could be fruitful. The results of our

study demonstrate that, during some years and for some locations, sampling outside of the recommended months is certainly productive. Thus, we recommend extending surveys through April and into May in years when breeding ponds do not fill until late winter (February-March).

The difference in dates of initial hatching and latest occupancy of flatwoods salamanders in 2005 and 2006 at Alpha pond at Fort Stewart was roughly 1.5 months. Of particular significance is the presence of larvae in a breeding pond until at least 23 May. Previously, the latest date that larvae had been observed at Fort Stewart was 13 April (1994), and that observation along with those from a day earlier were of larvae nearing metamorphosis (Gawin et al. 1995). The latest larvae capture dates of which we are aware are 1 May (1974) and 12 May (1972) (Williamson and Moulis 1979); these collections were made in Jasper Co., South Carolina. Although rarely observed, late occupancy is not necessarily a rare event; Williamson and Moulis (1979) captured larvae in the latter half of April or later in 4 consecutive years in South Carolina.

We do not know when the eggs were deposited that likely hatched 28 February 2005 because several rain events occurred in November and December of 2004 that could have triggered breeding migrations. The early January hatching date estimated for 2006 is probably not the earliest possible for this study site, because Anderson and Williamson (1976) observed eggs hatching as early as 4 December in southeastern South Carolina and southeastern Georgia. Little has been published regarding how long flatwoods salamander eggs (located in dry pond basins) remain viable after deposition before inundation, however, it is possible that larvae that hatched in late February 2005 were from eggs deposited 3 months earlier. Anderson and Williamson (1976) reported that advanced eggs taken from the field hatched in the laboratory approximately 74 days later. The terrestrially-deposited eggs of marbled salamanders, a related species, may remain viable 3-4 months post-oviposition (Noble and Brady 1933, Petranka and Petranka 1981). Flexibility in this aspect of reproduction is critical to flatwoods salamanders if rainfall during the reproductive season is below normal as it has been in southeastern Georgia for many of the last 10 years.

Our simple modeling of larval growth suggests that for the 2 years of our study a single date of hatching does not account for all the larval sizes observed. In 2005 we observed nearly dry ponds in mid-February and presumed that the larvae captured later in the spring hatched following significant rain events in late February or early March. However, the growth envelope initiated on 28 February does not include the largest larvae captured on 21 April which suggests that hatching also occurred on an earlier date. Four days of rain from 29 January to 3 February that totaled 25 mm was likely enough to partially fill the pond and could have inundated eggs at low pond elevations. Some larvae may have hatched during this partial pond filling and survived the following 3 weeks of minimal rain by taking refuge in small pools that remained. Alternatively, it is possible that we underestimated the actual larval growth rate in our model, but if that were the case, some larvae hatched on 28 February would have had to grow at a rate about 36% greater than the maximum rate estimated by Palis (1995). In 2006 the most likely date for hatching was in early January. However, hatching at that time does not account for all the sizes of larvae observed. We believe that some larvae must have also hatched in late January to account for the smaller individuals captured in early April.

Although we illustrated the two most likely hatch dates based on our analysis, we do not rule out the possibility that larvae hatched on more than two dates given the uncertainty in size at hatching and known variation in growth rates. It is quite possible that multiple hatching dates within a population during a season is the rule and not the exception. Since eggs are laid individually and not in large egg masses, multiple females would likely deposit their eggs at a variety of elevations within a dry depression. Gradual or incremental pond filling would therefore result in multiple hatching dates.

Of over 2,000 isolated depressional ponds on Fort Stewart, approximately 500 have been identified as potential breeding habitat for flatwoods salamanders (Palis 2002). Less than half of these ponds have been sampled to date, and most of those have not been sampled enough to conclude that they do not support breeding. Better knowledge of when larvae are present and most susceptible to specific sampling methods is crucial to successful monitoring and to maximize likelihood of detection. Sampling methods limit detectability during the first few weeks of larval residency because larval size is smaller than the mesh size of nets and traps. For example, in 2005 we thoroughly sampled Alpha pond twice during March without finding any larvae, but we found larvae in late April, and we are certain, based on the size of the larvae, that they were present throughout March.

4.4.4 Capture Rate – Abundance

We experienced capture rates of 0.012-0.66 larvae per minute in surveys in 10 different ponds. This is within the range of capture rates reported in a comprehensive review of 757 surveys in 176 ponds over 15 years (Bishop et al. 2006). They found reported capture rates ranging from 0.004 to 1 larvae per minute with most surveys reporting rates of >0.013.

We believe that the capture rate:density relationship we derived from the results of the removal studies is representative of most flatwoods salamander populations. Although it would be ideal to derive this relationship on a site-wide basis (such as for Fort Stewart), we believe this relationship can be used by others to at least generate an estimate of larval density for a sampled area based on capture rate. Obviously, one should also consider the relative quantity of the type of habitat sampled within a pond before making estimates of pond wide abundance.

Another useful value we derived was the average capture efficiency (# captured per # present) of about 40%. This means that effort at the level we performed in the enclosures (i.e., number of sweeps or time per area) is likely to capture 40% of the individuals in an area on average. That translates to a nearly 50:50 chance of detecting a larval salamander if even a single individual is present given that the level of effort is similar to what we employed.

4.4.5 Miscellaneous

This study was designed expecting to find flatwoods salamander larvae in several ponds at Fort Stewart. Method comparison results would have been averaged and compared across several ponds allowing us to make conclusions about the transferability of these results to other ponds at Fort Stewart and elsewhere. Unfortunately, we only found larvae at one pond at Fort Stewart and are forced to assume until future sampling shows otherwise that the one pond is representative of

others base-wide. We have no reason to believe that this is not the case. Fortunately, we were also successful at performing portions of the study at several sites in Florida. Similarly, until more sampling is done at other locations, we will have to assume that the relationship we derived between capture rate and larval density based on removal studies at St Marks is representative of sites elsewhere. Because we think that flatwoods salamander larval behavior with regards to how it normally rests in vegetative cover and how it might move when disturbed are fairly consistent range-wide, we believe that the catch relationships we derived should also be fairly consistent range-wide.

5. TASK 2: HABITAT ASSESSMENT

5.1 INTRODUCTION

Habitat models are an important tool for understanding the distribution of rare species and implementing management actions to conserve and restore their populations. Understanding the complex relationships between amphibians and the environments they inhabit will be critical in addressing global amphibian declines (Pechmann et al. 1991). In addition, conservation efforts for rare amphibians are increasingly concerned with the effects of changing conditions of local habitat patches and the spatial arrangement of habitat types on population viability (Biek et al. 2002). However, an accurate assessment of amphibian distributions is necessary for effective conservation planning and research on the ecology of amphibian populations and communities.

Habitat assessments for flatwoods salamanders have been problematic because the species is frequently known from only a few sites in a management area. This limits sample sizes for research, and pooling data from multiple areas across the range of the species may not improve the predictive capacity of models if wetland habitats are highly variable. In response to these issues, we developed habitat models for the flatwoods salamander at the Fort Stewart Military Reservation, Georgia, based upon knowledge of the life history of this species. We then tested these models at other flatwoods salamander breeding sites in Florida and explored the variation in wetland habitats across the range of the species to better understand the performance of our habitat models.

Habitat characteristics within or among different wetlands may be important in predicting the suitability of sites for flatwoods salamanders. Many amphibian larvae are susceptible to predators associated with permanent water and show a preference for breeding in temporary wetlands (Wellborn et al. 1997). Moreover, a study with tree frogs found that when adult frogs were presented with a choice of artificial ponds that contained or did not contain fish, they showed a strong preference for ponds without fish (Binckley and Resitarits 2002). Since wetland plants vary in their tolerances for flooded conditions, it is possible that wetland plant species may reflect wetland hydroperiods and improve our ability to predict where flatwoods salamanders breed. Within wetlands that have a similar hydroperiod, canopy cover may also affect the fitness of larval amphibians. Thurgate and Pechmann (2007) demonstrated that competitive interactions between amphibians may be influenced by canopy cover, and other studies have documented shifts in amphibian communities associated with canopy cover at ponds that result from effects on larval growth (Skelly et al. 2002).

Landscape structure may also improve our ability to predict the suitability of wetland breeding sites for flatwoods salamanders. Metapopulation theory predicts that occupancy of breeding sites should be associated with proximity to other suitable habitat and the ability of individuals to move through the landscape, and studies of amphibians support both of these predictions. Amphibian populations show an increased likelihood of using breeding ponds that were in closer proximity to adjacent occupied ponds (Gulve 1994), and in some cases local habitat conditions appear to be less important in determining amphibian distributions than population processes (Schmidt and Pellet 2005). Moreover, studies of amphibian movement indicate that some

landscape features benefit amphibian populations by increasing survivorship and facilitating movement (Mazerolle 2005), while juvenile dispersal is associated with habitats that reduce the risk of desiccation (Rothermel and Semlitsch 2002). At a community level, larval amphibians may have strong interactions with predators (Wilbur 1997). Many larval amphibians that develop in temporary wetlands suffer reduced survivorship and growth rates in the presence of predators like fish that are associated with permanent water (Gregoire and Gunzberger 2008). However, studies of fish populations in temporary wetlands in the southeastern coastal plain have found that wetland position is more important than wetland habitat in predicting fish presence (Baber et al. 2002, Sun et al. 2001, Snodgrass et al. 1996), so wetland position within a landscape may be important in predicting the use of sites by flatwoods salamanders.

Our analysis focuses on two fundamental tasks related to the conservation of a rare amphibian. First, we use knowledge of amphibian ecology to test competing models for predicting the presence and absence of flatwoods salamanders. Second, we quantify the similarity among sites from across the range of the species. While predicting the presence of a rare species is a common task, predicting sites where a species is absent may be just as important for management purposes. In addition to understanding where a species is likely to be present or absent, obtaining a broad perspective about the variation of habitat across the range of a species is important if habitat models are to be extrapolated to areas that have not been as extensively sampled. Failure to appreciate the variation in habitat across a species range could lead to inappropriate application of models to areas with different environmental conditions. Moreover, understanding the variation in potential habitats reveals whether areas sampled for a species are representative of other sites that have yet to be surveyed (Hargrove et al. 2003). We used logistic regression and linear discriminant functions to predict the presence and absence of flatwoods salamanders from breeding sites, and we used hierarchical clustering to explore the variation in habitat across the range of the species.

5.2 METHODS

5.2.1 Study Areas

We compiled locality data for the flatwoods salamander from Fort Stewart, Georgia, and two additional sites in the Florida panhandle: the Apalachicola National Forest and St Marks National Wildlife Refuge. Fort Stewart contains flatwoods salamander breeding sites in both wet flatwoods and sandhill landscapes. The Apalachicola National Forest contains flatwoods salamander breeding sites primarily in wet flatwoods and savannah landscapes, and breeding sites at St. Marks National Wildlife Refuge are found in depressional wetlands dominated by sawgrass.

5.2.2 Amphibian Surveys

We conducted dipnet surveys for larval flatwoods salamanders and other aquatic amphibians between January and May of each year from 2005 – 2007 at 81 wetland sites on Fort Stewart, and only found flatwoods salamander larvae at one pond during this period as previously

described. As a result of our limited occurrence data for this species, we decided to pool data from larval surveys conducted for flatwoods salamanders between 1994 and 2007.

Presence was determined if the flatwoods salamander had been documented in a wetland site between 1994 and 2007, and absence was based upon expert opinion about wetlands being unsuitable for breeding due to the permanence of water or vegetation (personal communication, D. Stevenson). Thus, while presence and absence can be viewed as being opposite extremes in habitat suitability, they are not complements of one another: our set of wetlands included some sites that were not classified as having presence or absence of our focal species.

This resulted in the selection of 81 survey sites for our study, with 14 of these being documented flatwoods salamander breeding sites, 16 being considered unsuitable for flatwoods salamander breeding, and 51 considered as having some potential as flatwoods salamander breeding sites. We also selected additional sites in the Apalachicola National Forest and St. Marks National Wildlife Refuge for the purpose of testing our habitat models developed with data from Fort Stewart. Twenty-one of these sites were known to have had flatwoods salamander breeding activity between 2002 and 2005, while the remaining 29 sites were considered to be potential flatwoods salamander breeding sites.

5.2.3 Wetland Vegetation Data

We sampled wetland vegetation along two perpendicular transects within wetlands, one north to south, and one east to west. Occasionally, the pond would be laid out such that you could not get a good sample from E-W and N-S, so other directions were used, but the two transects always bisected each other at a right angle in the middle of the pond. Transects were started at the edge of the wetland basin based upon the vegetation in the ecotone.

Presence or absence measurements were taken of the canopy and ground cover every five steps (approximately every 4 meters) along the transect. To estimate canopy and ground cover, we looked up and down at each stop and recorded a "+" if there was vegetation within the crosshairs of a 4-cm diameter paper tube, and a "-" if there was sky (in the canopy) or open water or bare ground (on the ground). The number of measurements taken along transects ranged from 15 to 40.

At every third stop, ground cover estimates of bare ground, water, and herbaceous and emergent wetland plants were taken within a 1-m radius, and percent shrub and small tree cover was estimated within a 5-m radius. At every third stop, vegetation types and their percent cover within a 1-m radius circle were noted. Types of vegetation were listed, and their percent cover was estimated along with the amount of open water within the circle. Shrub and small tree cover were estimated within a 5-m radius. At the beginning and end of each transect, a percent cover estimate was taken of the grasses, shrubs, and trees in the upland habitat. Plant species included in our characterization are listed in Table 7. A list of all ponds whose vegetative habitat was characterized during the course of the study and the years each was sampled is included in Appendix A.3.

Table 7. Plant species identified during habitat characterization at Fort Stewart ponds and their corresponding wetland indicator status

Plant species	Common name	Wetland indicator status
Andropogon virginicus	broomsedge	facultative
Carex atlantica	prickly bog sedge	facultative wetland
Dichanthelium spp.	rosette grass	facultative
Ilex coriacea	gallberry	facultative wetland
Gratiola spp.	hedgehyssop	status variable
Eriocaulon spp.	pipewort	obligate
Ilex opaca	American holly	facultative
Hypericum spp.	St. Johns-wort	status variable
Iris tridentata	savannah iris	obligate
Ludwigia spp.	primrose-willow	obligate
Lyonia lucida	fetterbush	facultative wetland
Proserpinaca palustris	mermaidweed	obligate
Myrica cerifera	waxmyrtle	facultative
Panicum virgatum	switchgrass	facultative
Pontederia cordata	pickerelweed	obligate
Sarracenia spp.	pitcher plant	obligate
Toxicodendron radicans	poison ivy	facultative
Persea borbonia	redbay	facultative wetland
Rhynchospora spp.	beakrush	status variable
Rubus spp.	blackberry	status variable
Sagittaria spp.	arrowhead	obligate
Onoclea sensibilis	sensitive fern	facultative wetland
Smilax spp.	greenbriar	facultative
Xyris spp.	yellow-eyed grass	obligate
Sphagnum spp.	sphagnum	status variable
Aristida spp.	wiregrass	status variable
Sporobolis spp.	dropseed	status variable
Hydrocotyle spp.	pennywort	status variable
Cyrilla racemiflora	Titi	facultative wetland
Lycopodium spp.	clubmoss	status variable
Bacopa spp.	waterhyssop	status variable
Pinguicula spp.	butterwort	status variable
Cladium spp.	sawgrass	obligate
Osmunda regalis	royal fern	obligate
Taxodium distichum	cypress	obligate
Nyssa sylvatica	black gum	facultative
Acer rubrum	red maple	obligate
Ilex myrtifolia	myrtle leaf holly	facultative wetland
Pinus elliotii	slash pine	facultative wetland
Quercus nigra	water oak	facultative
Liquidambar styraciflua	sweetgum	facultative

Plant species were identified to the lowest possible taxonomic unit. For habitat modeling, we also grouped plants according to both structural characteristics and their wetland indicator status. Plants were placed into one of the following structural groups: graminaceous vegetation, emergent vegetation, submerged aquatic vegetation, ferns, shrubs, and trees. Plants were also placed into one of the following wetland indicator status categories designated by the U.S. Fish and Wildlife Service (1988): obligate, facultative wetland, facultative, and upland. Wetland indicator status reflects the likelihood that a plant occurs in a wetland and does not reflect the degree of wetness at a site.

5.2.4 GIS Data

We compiled spatial data on the proximity and amount of different landscape features (i.e., wetlands, streams, roads and land cover) within 2 km of wetlands selected for surveys into a geographic information system (GIS). We selected a buffer of 2 km based upon movement data of congeneric salamanders and other amphibians (USFWS 1999, Smith and Green 2005). We obtained National Wetlands Inventory (NWI) Data in polygon format at a scale of 1:24,000 from the USFWS. Wetlands were identified as being temporary or permanent based upon their NWI category. We calculated the distance to the nearest permanent and temporary wetland as well as the total area of permanent and temporary wetlands within the buffer for each site.

We also used road and stream data in a polyline format. Road data were obtained from the Department of Public Works at Fort Stewart. For each wetland site we calculated the distance to the nearest road as well as the total length of roads within the 2-km buffer. Although Fort Stewart has both paved and unpaved roads, roads off of the cantonment area are primarily unpaved with the exception of the major county highways that cross the base. We did not tabulate distances based on road type in this analysis. We obtained stream data at a scale of 1:24,000 from the U.S. Geological Survey. The distance to the nearest stream and the total length of streams within the 2-km buffer were calculated.

Finally, we used the National Land Cover Data from the U.S. Environmental Protection Agency in linear discriminant functions. We did not use this data set in logistic regression models because of concerns about differences in the evergreen forest among different management areas. These data were available in a raster format with a pixel size of 30 x 30 m. We clipped the raster with the 2-km buffer for each wetland site.

The vegetation and landscape data used in the habitat modeling is provided electronically with this report. The accompanying metadata that describes the data file is included in Appendix A.4.

5.2.5 Data Analysis

We used logistic regression and linear discriminant functions to predict whether sites should be occupied by flatwoods salamanders and hierarchical clustering to quantify the variation in habitat across the range of the species. Prior to conducting analyses, we checked variables for normality and applied appropriate transformations when necessary. We also screened variables for high correlations ($r > 0.7$). To avoid problems associated with collinearity, we retained the variable with the greatest among-group variance when variables were strongly correlated (McGarigal et

al. 2000). We also tested for homogeneity of variance with Levene's test (McGarigal et al. 2000). Finally, we standardized data prior to our analysis to account for the different scales of variables.

Logistic regression was performed separately for both presence and absence of the flatwoods salamander. We used forward and backward stepwise variable selection to identify models that fit the data well, and we compared these models with an *a priori* model for salamander presence that included 3 plants that are often associated with the presence of flatwoods salamanders by herpetologists: wiregrass (*Aristida* spp.), hatpin (*Eriocaulon* spp.), and beakrush (*Rhyncospera* spp.). We ran stepwise selection procedures on wetland vegetation and landscape data separately. Model selection criteria were evaluated, and the parameters from the best models in each category were then combined into a new set of variables that had forward and backward stepwise regression procedures performed on it. We then compared these models with models for presence and absence of flatwoods salamanders based upon wetland vegetation and landscape structure.

We expected that some variation in plant species across the range of the flatwoods salamander might make models from Fort Stewart perform poorly with data from Florida, so we analyzed wetland vegetation in three ways. First, we used cover estimates of different plant species in logistic regression analyses. Then we grouped cover estimates of plant species by type of vegetative structure (i.e., tree, shrub, herbaceous vegetation, emergent aquatic vegetation, submerged aquatic vegetation). Finally, we grouped cover estimates of plant species by wetland indicator status. We anticipated that grouping similar types of plant species might improve model performance, for example, if different plant species in Georgia and Florida provided a similar structural component in salamander habitat, then models with structural components might perform better than models with plant species. We evaluated logistic regression models in two ways after comparing model selection criteria. First, we compared the predicted probabilities of presence or absence at ponds at Fort Stewart or in both the Apalachicola NF and St Marks NWR that were known to contain or lack flatwoods salamanders. Second, we compared the predicted probability of presence or absence in ponds at Fort Stewart with an expert assessment of pond suitability.

Linear discriminant functions were also used to predict the presence and absence of the flatwoods salamander in wetland sites and reveal relationships among habitat variables at Fort Stewart. We generated discriminant functions with 2 sets of data: wetland vegetation data, and landscape data. We assessed the predictive accuracy of discriminant functions by examining the predicted classification of sites with known, suitable, and unsuitable ponds. Although we tested for normality of individual variables included in our analysis, we did not test for multivariate normality directly. While linear discriminant functions are somewhat robust to a lack of multivariate normality, stepwise variable selection is less so, thus we did not perform stepwise selection with linear discriminant functions (McGarigal et al. 2000). Moreover, linear discriminant functions reveal how different variables are related to one another, so performing variable selection in discriminant functions would limit our ability to determine how variables are related to one another and to the presence or absence of flatwoods salamanders.
Finally, we used hierarchical clustering to analyze the variation in wetland vegetation and landscape characteristics associated with wetland sites in Florida and Georgia. Hierarchical

clustering begins by assigning each sampling site a unique group. The unique groups that are most similar are then grouped together, and this process proceeds by combining similar groups into new clusters until all groups are included in one large cluster. We used single, complete, average, and Ward's linkages to generate dendrograms (McGarigal et al. 2000), and we presented the dendrogram resulting from the single linkage after verifying that it was corroborated by the dendrograms produced with other linkages. Hierarchical clustering produces a dendrogram in which the most similar sites are contained on adjacent branches. We anticipated that the patterns in the dendrograms would fall into one of two categories. First, sites with a similar status might group together regardless of whether they were in Florida or Georgia, which would indicate that known breeding locations have more in common with each other than they do with unsuitable sites. Second, if significant geographical variation in habitat exists then sites from Georgia would be more similar to each other than they are to sites in Florida.

Because we used what little information was available on salamander residency in Fort Stewart ponds to train the occupancy models, we were not able to test which model is the best predictor of occupancy for unknown ponds. Since the different models produced different results, we chose to combine the results for the purpose of supplying Fort Stewart with a priority list of ponds for future sampling. A cumulative index was derived from a sum of rank scores assigned to the results of three models: 1) logistic regression: Landscape+Plant species; 2) logistic regression: Wetland plants model; and 3) discriminant function classification model. Using the results of the first two models, ponds were ranked in quintiles (fifths) with the highest quintile assigned a score of 1 and the lowest a score of 0; ponds in intermediate quintiles received scores of 0.75, 0.5, and 0.25, respectively. Since the discriminant function model just predicted presence or absence, the two outcomes were scored 1 for presence and 0 for absence. The scores for the three models were added for a maximum possible score of 3.

5.3 RESULTS

5.3.1 Logistic Regression

Two models based upon wetland vegetation predicted presence of flatwoods salamanders well (Table 8). Different models were evaluated based on their Akaike information criterion (AIC) which compares models based on the measure of fit to the data and the number of parameters in the model with fewer parameters (i.e., less model complexity) scoring better. The model based upon wetland indicator status of plants performed slightly better than the model based upon plant species alone. Our *a priori* model of presence being related to hatpin, beakrush, and wiregrass did not perform as well as the alternative models produced by the stepwise selection procedure. The average predicted probability of presence for known salamander breeding sites was only 34%, but known breeding sites from Florida were predicted to have a much lower probability of occupancy by both of these models (Figure 22). Models based upon wetland vegetation alone predicted similar probabilities of occupancy for the ponds ranked as unlikely breeding sites by experts (Figure 23). We did not combine parameters from different vegetation models (e.g., plant species plus wetland indicator species) because doing so would have introduced collinear terms into the analysis.

Landscape data did not predict presence of flatwoods salamanders well on its own, but combining landscape data (in particular, the distances to the nearest road and stream) with plant species significantly improved the fit of models for salamander presence in ponds (Table 8). Predicted probabilities of presence were highest in known and suitable ponds, while predicted probabilities of absence were highest in unsuitable ponds (Figure 23).

Table 8. Logistic regression models for predicting presence and absence of the flatwoods salamander at potential breeding sites on Fort Stewart. Better models have lower AIC scores

Model Components	# Parameters	AIC
…for Presence		
Landscape + Plant Species[a]	4	62.83
Wetland Indicator Status[b]	2	64.61
Plant Species[c]	4	66.37
Landscape[d]	2	69.74
Landscape + Wetland Indicator Status[e]	4	70.87
Hatpin + Beakrush + Wiregrass	3	72.62
Plant Structure[f]	1	72.93
…for Absence		
Wetland Indicator Status[g]	3	63.04
Plant Structure[h]	2	64.79
Plant Species[i]	3	74.04
Landscape + Plant Species[j]	6	85.60
Landscape + Wetland Indicator Status[k]	4	90.03
Landscape[l]	2	98.29

[a] Distance to Stream + Distance to Road + Iris + Sweetgum
[b] Facultative + Obligate
[c] Iris + Dichanthelium + Sensitive Fern + Sweetgum
[d] Distance to Stream + Distance to Road
[e] Distance to Stream + Distance to Road + Facultative + Obligate
[f] Tree
[g] Facultative + Facultative Wetland + Obligate
[h] Aquatic + Tree
[i] Dichanthelium + Ludwigia + Sensitive Fern
[j] Distance to Road + Distance to Stream + Dichanthelium + Ludwigia + Sensitive Fern
[k] Distance to Road + Distance to Stream + Facultative + Obligate
[l] Distance to Road + Distance to Stream

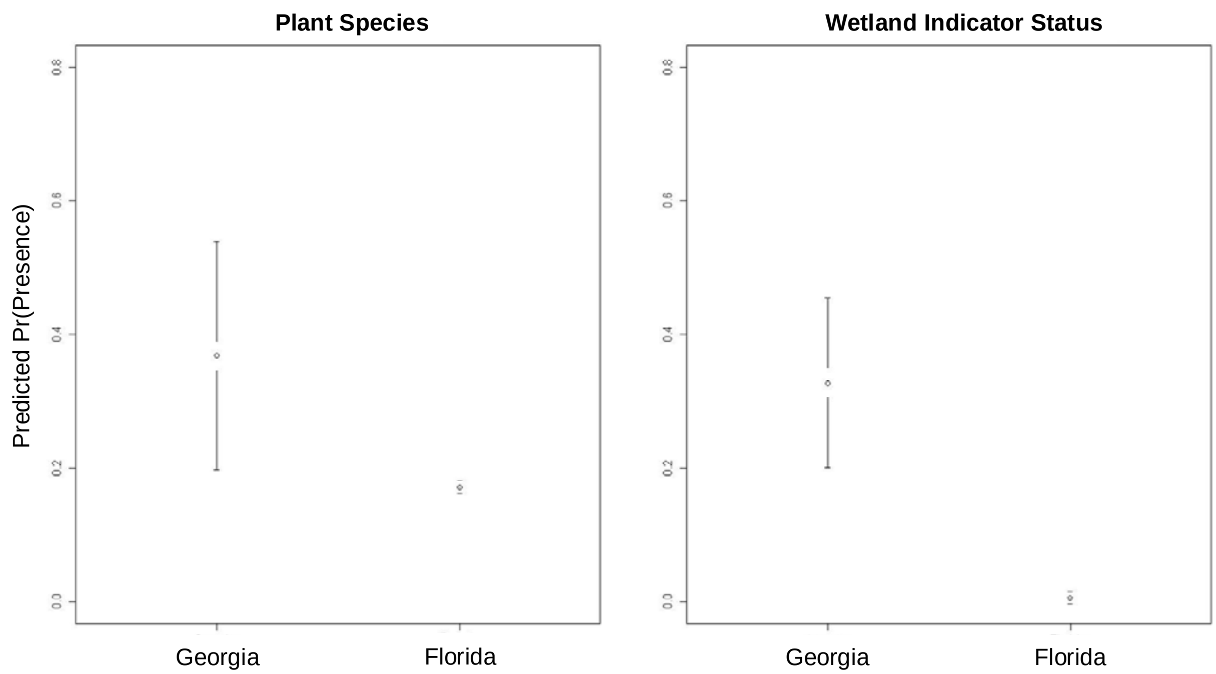


Fig. 22. Predicted probabilities of occupancy for known flatwoods salamander breeding sites in Georgia (Fort Stewart) and Florida (Apalachicola NF and St. Marks NWR) from logistic regression models based upon plant species and wetland indicator status.

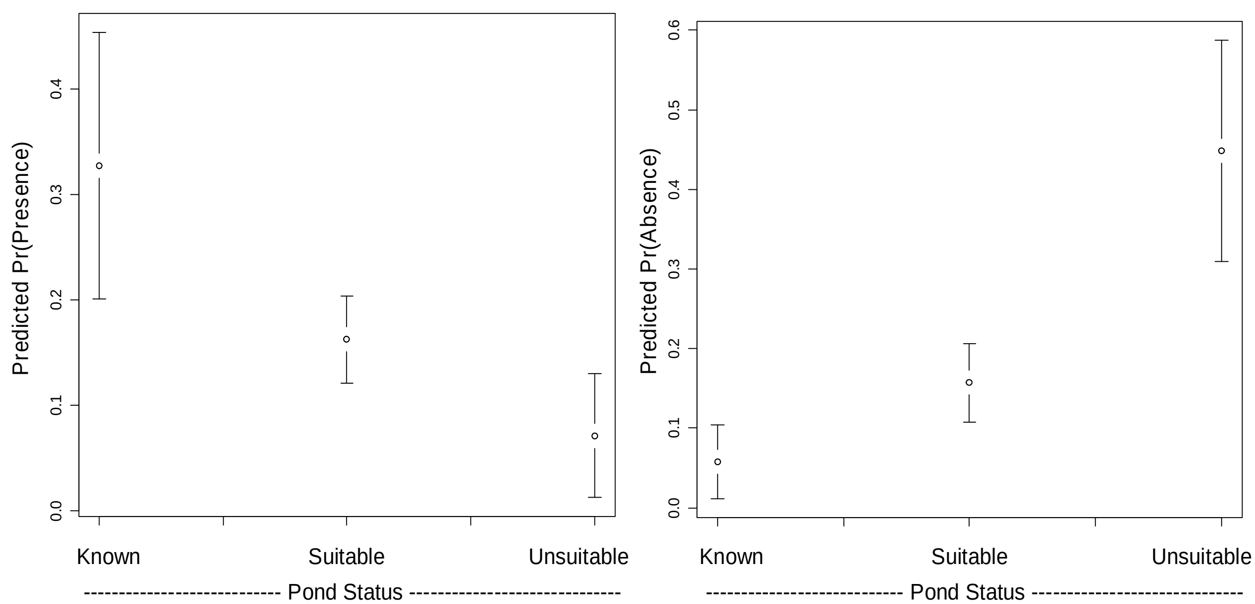


Fig. 23. Predicted probabilities (mean ± 95% confidence interval) of presence and absence for potential flatwoods salamander breeding sites at Fort Stewart based on logistic regression analysis.

5.3.2 Linear Discriminant Functions

Linear discriminant functions indicated that wetland vegetation data did slightly better at classifying sites than landscape data, but both discriminant functions had low rates of misclassification. Linear discriminant functions with wetland vegetation data correctly classified all 14 known breeding sites as having flatwoods salamanders present and 15 unsuitable breeding sites as having flatwoods salamanders absent, while linear discriminant functions with landscape data misclassified 1 of the 15 unsuitable sites and 2 of the 14 known breeding sites (Table 9). Both the discriminant function based on landscape data and discriminant function based on wetland vegetation showed good separation between the groups with individual variables (Tables 10 and 11). Presence was most strongly associated with lower amounts of temporary and permanent wetlands in the surrounding landscape and greater distances to permanent wetlands (Table 10), while relationships between presence and individual plants species varied (Table 11).

Table 9. Classification of known and unsuitable wetlands by linear discriminant functions with landscape and wetland vegetation data

	Discriminant Function Classification			
	Wetland Vegetation Data		Landscape Data	
Actual Status	**Present**	**Absent**	**Present**	**Absent**
Known	14	0	12	2
Unsuitable	0	15	1	14

Table 10. Group means and coefficients for variables included in the linear discriminant function to predict flatwoods salamander presence or absence with landscape data

Parameter	Coefficient	Group Mean	
		Larvae Absent	**Larvae Present**
Stream Length	0.5414631	-0.1014843	0.1087332
Road Length	-0.6183600	-0.1199976	0.1285688
Distance to Nearest Road	0.0988086	-0.2302107	0.2466544
Distance to Nearest Stream	0.3129034	-0.0357811	0.0383369
Distance to Nearest Permanent Wetland	-0.7794745	0.1427152	-0.1529092
Distance to Nearest Temporary Wetland	-0.3967716	0.0669362	-0.0717174
Area of Permanent Wetlands	-0.7542532	0.1022731	-0.1095783
Area of Temporary Wetlands	-1.1992045	0.3361464	-0.3601569

Table 11. Group means and coefficients for variables included in the linear discriminant function to predict flatwoods salamander presence or absence with wetland vegetation data

Parameter	Coefficient	Group Mean	
		Absent	Present
Cypress	2.56033125	0.15239494	-0.1632803
Dichanthelium	-2.09496502	0.31127385	-0.3335077
Gratiola	-3.60813126	-0.1856953	0.1989593
Gum	-1.36910869	-0.6841754	0.733045
Hatpin	2.27590522	0.06068571	-0.0650204
Holly	0.9894831	0.12700663	-0.1360785
Hypericum	-3.417678	0.37385113	-0.4005548
Ilex	0.69077241	-0.4195648	0.4495337
Blueflag	2.9596684	-0.3001583	0.3215981
Ludwigia	-3.6205598	-0.3578164	0.3833747
Lyonia	-2.13537917	0.3445415	-0.3691516
Maple	-0.29930536	-0.2600522	0.2786274
Mermaidweed	-2.0446887	0.0966194	-0.1035208
Panicum	3.44686641	0.08504068	-0.091115
Pickerelweed	3.14226953	-0.0390276	0.0418152
Redbay	0.94571809	-0.1951835	0.2091251
Rhyncospera	3.89684212	-0.0451823	0.0484096
Rubus	3.04141047	-0.2630684	0.281859
Rush	1.31457355	0.0730235	-0.0782395
Sensitive fern	-3.84532961	0.34241999	-0.3668786
Slash Pine	5.51950622	-0.219135	0.2347875
Greenbriar	0.25456788	0.14840696	-0.1590075
Snakehead	-0.03535999	-0.0780733	0.08365
Sphagnum	-0.22139395	-0.0237464	0.0254426

5.3.3 Cluster Analyses

The cluster analysis reveals two general patterns in wetland vegetation at flatwoods salamander sites. First, habitat characteristics are most similar among wetland sites from the same area; the Florida and Georgia sites are distinctly different. Second, known breeding sites tend to be most similar to other suitable breeding sites rather than unsuitable breeding sites (Figure 24). These patterns were consistent with all four of the different linkages used.

A cluster analysis based on landscape features of all previously known flatwoods salamander locations identifies those areas with similar landscape characteristics. The area identified closely approximates the known range of flatwoods salamanders (Figure 25). When using a small subset of known location to train the model, we found that known sites in the Florida panhandle are most similar to potential habitat in the western Florida panhandle and southern Alabama (Figure 26), while known sites at Fort Stewart are most similar potential habitat in northeastern Florida, Georgia and South Carolina (Figure 27).

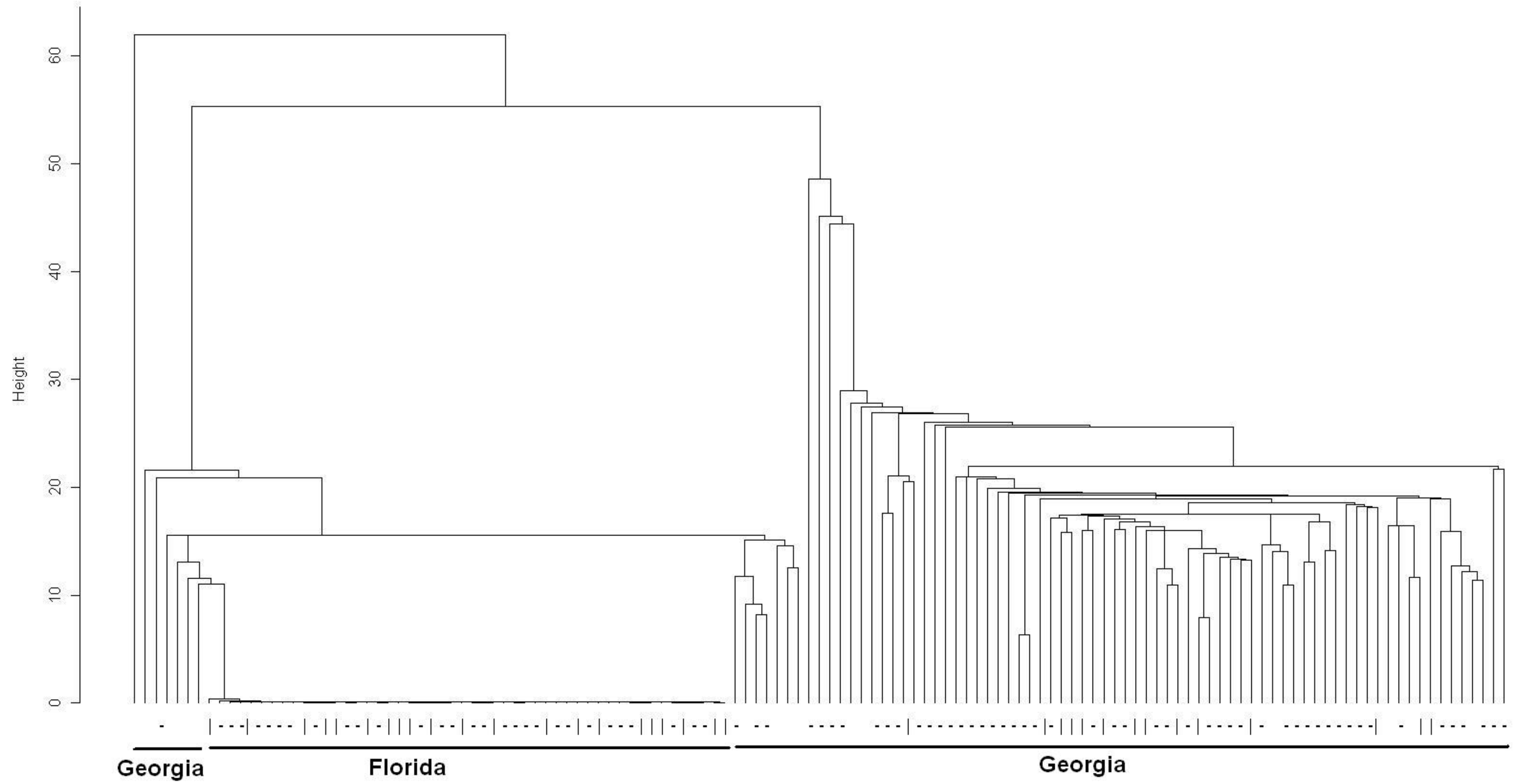


Fig. 24. Cluster analysis of vegetation at known (|), suitable (-), and unsuitable (blank) wetlands in the Apalachicola National Forest (Florida), Fort Stewart (Georgia), and St Marks National Wildlife Refuge (Florida). Height of lines connecting ponds is an arbitrary measure of similarity among ponds.

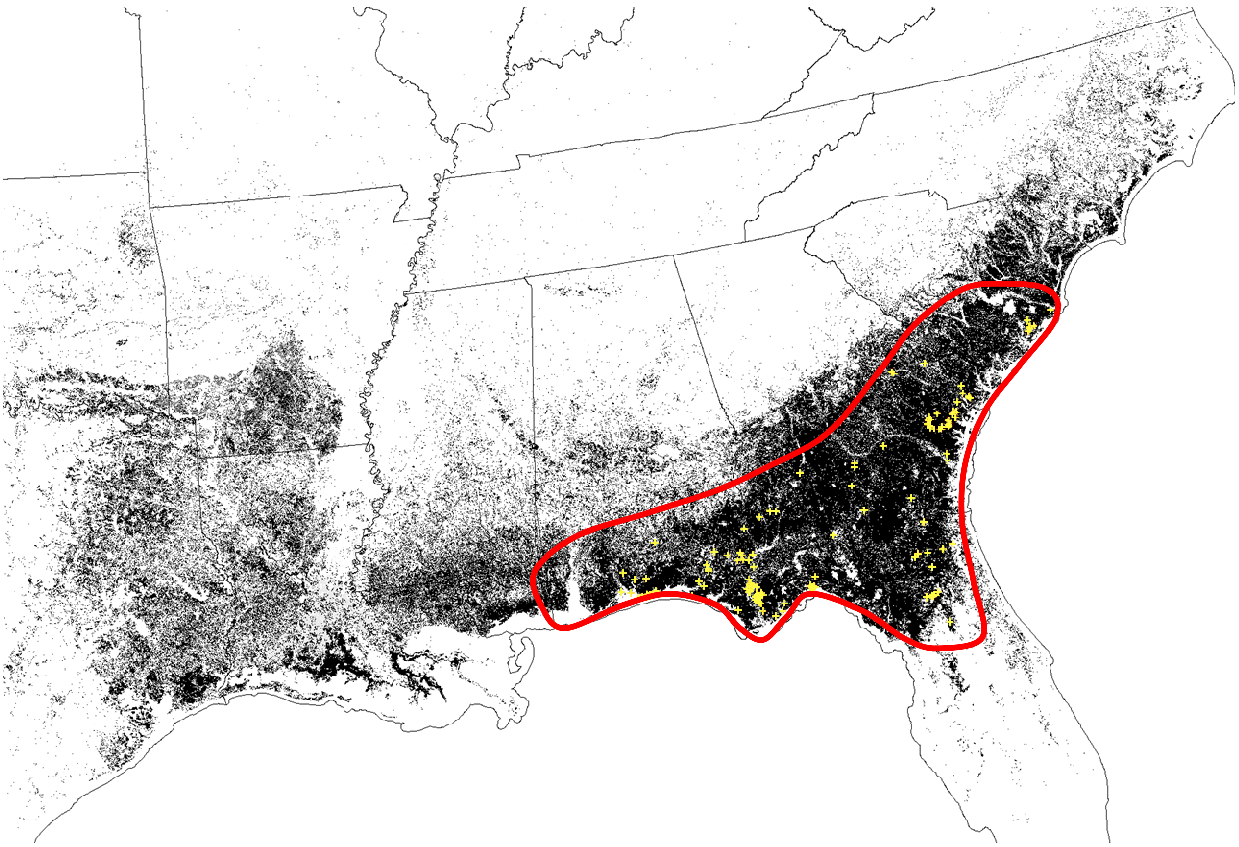


Fig. 25. Hierarchical clustering shows the extent of the known ranges of the flatwoods salamanders (outlined in red) contains landscape-level habitat that is similar to known locations from across the range (yellow crosses).

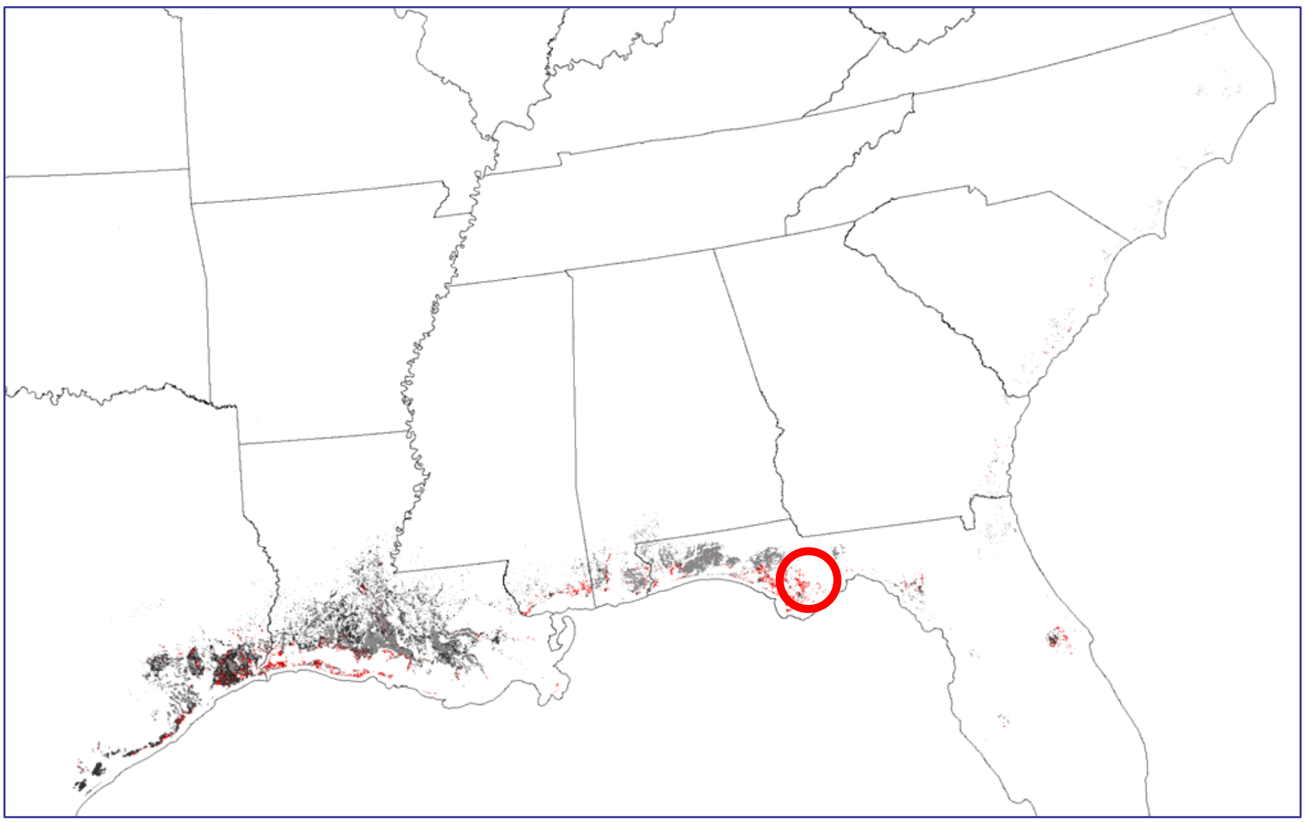


Fig. 26. Hierarchical clustering shows landscape-level habitat in the western Florida panhandle and southern Alabama are the most similar to sites from the Apalachicola National Forest (circled in red).

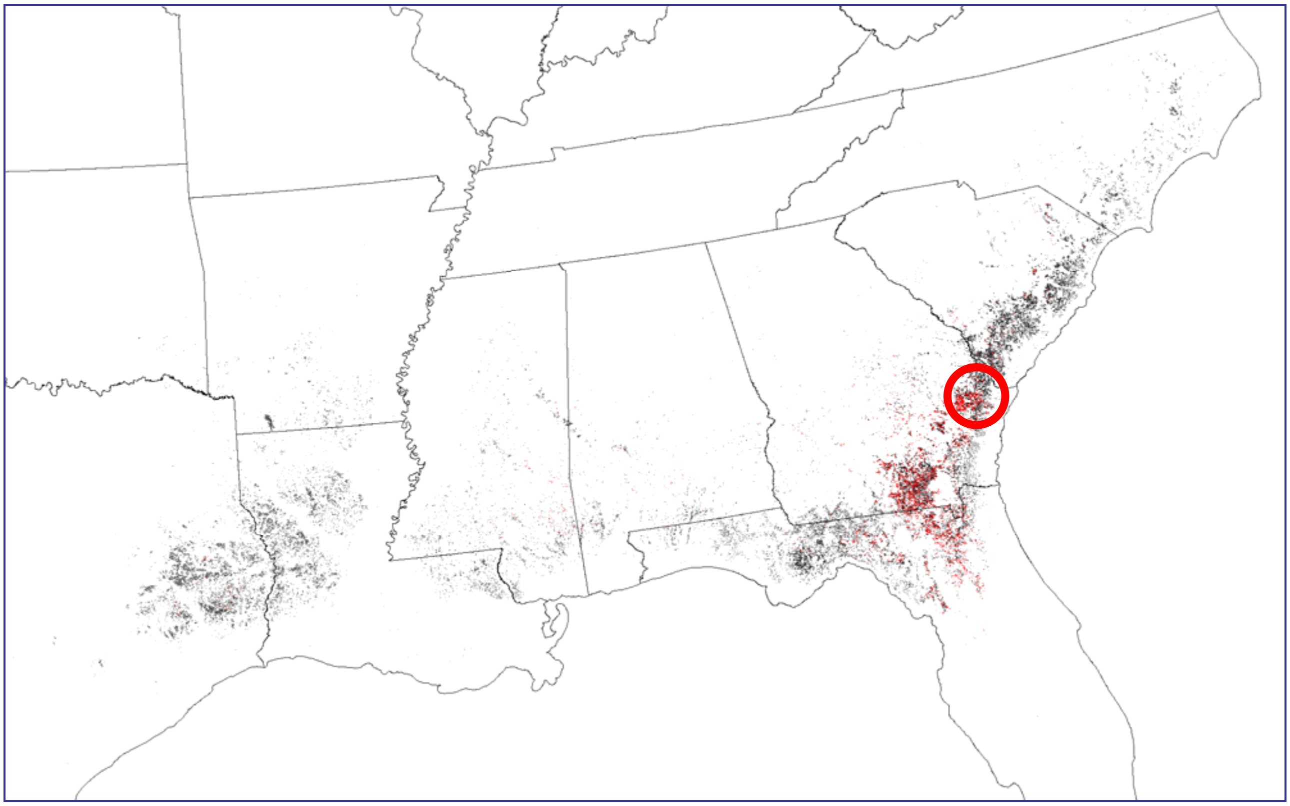


Fig. 27. Hierarchical clustering shows landscape-level habitat at Fort Stewart (sites circles in red) is most similar to other areas from the known range of the flatwoods salamander in northeastern Florida, Georgia, and South Carolina.

5.4 FORT STEWART POND PRIORITIZATION

The results of the three habitat statistical models were combined in a single index score in order to rank 80 ponds at Fort Stewart based on their similarity to ponds that were historically known breeding sites (Table 12). The 17 previously confirmed breeding ponds had scores ranging from 2 to 3 (Figure 28). We recommend that those ponds ranked along with the previously known ponds (scores of 2 to 2.75) be considered highest priority for future sampling. A second tier of ponds should be those scoring 1 to 1.75, and those scoring below 1 should be of low priority.

Table 12. Cumulative index derived from the results of various methods for predicting likelihood of flatwoods salamander occurrence in 80 Ft Stewart ponds

Pond	P&S subjective ranking	Logistic regression: Landscape+ Plant species model	Logistic regression: Wetland plants model	Logistic regression: Plant species model	Discriminant function classifica-tion model	Cumulative index
A10.2-02	1	0.369	0.345	0.301	1	3
A4.2-09	1	0.369	0.255	0.518	1	3
A6.4-03	1	0.304	0.621	0.274	1	3
F7.2-02	1	0.51	0.269	0.362	1	3
F9.5-01	1	1	0.937	1	1	3
A10.2-01	1	0.169	0.358	0.171	1	2.75
A5.3-04	3	0.2	0.266	0.171	1	2.75
A6.4-05	1	0.415	0.231	0.272	1	2.75
A7.1-02	3	0.282	0.253	0.333	1	2.75
E10.3-06	3	0.238	0.525	0.169	1	2.75
F9.5-02	1	0.143	0.466	0.171	1	2.75
A10.2-07	1	0.238	0.228	0.171	1	2.5
A5.1-01	3	0.206	0.208	0.171	1	2.5
A6.4-02	1	0.986	0.145	0.963	1	2.5
A7.1-04	3	0.51	0.121	0.446	1	2.5
A8.0-03	3	0.484	0.13	0.652	1	2.5
F6.4-04	2	0.129	0.419	0.16	1	2.5
F6.5-04	2	0.192	0.196	0.163	1	2.5
F7.2-01	2	0.137	0.371	0.093	1	2.5
F7.6-01	1	0.663	0.11	0.571	1	2.5
E11.2-02	1	0.198	0.177	0.167	1	2.25
F6.4-09	2	0.127	0.192	0.151	1	2.25
A6.4-01	5	0.38	0.327	0.274	0	2
E10.3-04	1	0.063	0.237	0.062	1	2
E11.2-05	2	0.271	0.098	0.161	1	2
F6.3-04	1	0.077	0.204	0.145	1	2
F6.3-06	3	0.119	0.119	0.171	1	2
F6.3-07	2	0.125	0.153	0.171	1	2
F6.7-12	3	0.441	0.005	0.272	1	2
A6.1-03	3	0.286	0.237	0.352	0	1.75
A7.1-01	5	0.165	0.293	0.171	0	1.75
D14.2-01	3	0.125	0.104	0.171	1	1.75
D14.2-02	2	0.177	0.013	0.171	1	1.75
D14.2-07	3	0.119	0.082	0.171	1	1.75
F6.3-03	2	0.041	0.165	0.109	1	1.75
F6.5-05	3	0.067	0.144	0.091	1	1.75
A5.1-02	5	0.404	0.148	0.28	0	1.5
A7.1-05	3	0.243	0.236	0.171	0	1.5
A8.0-02	2	0	0.137	0	1	1.5
D14.2-06	3	0.087	0.514	0.143	0	1.5

Pond	P&S subjective ranking	Logistic regression: Landscape+ Plant species model	Logistic regression: Wetland plants model	Logistic regression: Plant species model	Discriminant function classifica-tion model	Cumulative index
E10.3-10	3	0.138	0.036	0.086	1	1.5
F6.2-03	3	0.045	0.088	0.052	1	1.5
F6.2-05	3	0.071	0.065	0.05	1	1.5
A6.1-04	3	0.142	0.203	0.226	0	1.25
A6.1-05	3	0.319	0.098	0.439	0	1.25
F6.3-02	2	0.057	0.714	0.171	0	1.25
F6.3-05	2	0.087	0.207	0.162	0	1.25
A6.1-06	3	0.089	0.14	0.171	0	1
D12.1-03	2	0.019	0.221	0.05	0	1
D12.1-04	3	0	0.007	0	1	1
D12.3-01	3	0.145	0.051	0.171	0	1
E10.3-01	3	0.064	0.191	0.046	0	1
F6.1-01	2	0.242	0.08	0.151	0	1
F6.2-02	4	0	0.411	0	0	1
F6.2-06	3	0.118	0.111	0.076	0	1
F7.2-05	2	0.035	0.231	0.035	0	1
A6.2-04	5	0.107	0.037	0.251	0	0.75
D12.3-08	2	0.063	0.12	0.137	0	0.75
E11.3-01	5	0.201	0.004	0.171	0	0.75
F6.2-08	5	0	0.213	0	0	0.75
F6.3-08	2	0.084	0.115	0.086	0	0.75
F6.4-10	2	0.07	0.123	0.11	0	0.75
F6.5-02	3	0.14	0.088	0.156	0	0.75
F7.2-07	3	0.155	0.004	0.159	0	0.75
D13.1-01	5	0.089	0.004	0.104	0	0.5
E11.2-07	3	0.05	0.048	0.048	0	0.5
F6.5-14	3	0.067	0.104	0.071	0	0.5
A5.1-03	5	0	0.04	0	0	0.25
D12.1-01	3	0.001	0.048	0.004	0	0.25
D12.1-02	2	0.032	0.004	0.061	0	0.25
D14.2-08	3	0	0.037	0	0	0.25
F6.5-03	3	0	0.041	0	0	0.25
D12.3-02	5	0	0.004	0	0	0
D13.1-02	5	0	0.005	0	0	0
D14.2-11	5	0	0.021	0	0	0
E10.2-02	5	0.017	0.009	0.022	0	0
E10.2-03	5	0.011	0.005	0.016	0	0
E10.3-08	6	0.005	0.006	0.007	0	0
E10.3-11	5	0.011	0.008	0.012	0	0
F6.2-01	4	0	0.005	0	0	0

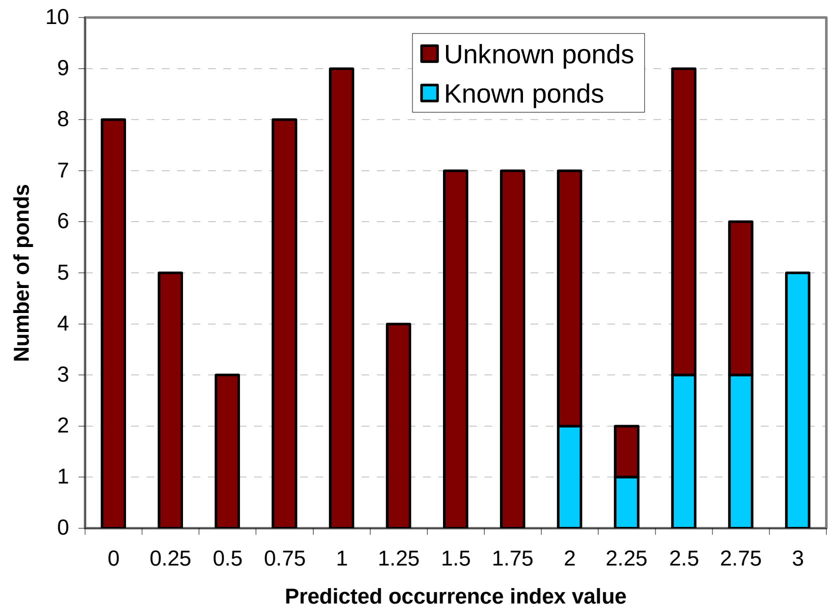


Fig. 28. Distribution of predicted occurrence index scores for 60 Fort Stewart ponds derive from results of three habitat models.

5.5 DISCUSSION

Our results from modeling flatwoods salamander habitat at Fort Stewart are corroborated by prior studies that have identified both landscape and wetland vegetation features associated with pond-breeding amphibians. First, we found that the presence of flatwoods salamanders was positively associated with a native iris species and the presence of facultative and obligate wetland plants. This is consistent with a previously published study that found an association between herbaceous vegetation, fire frequency, and wetland use by flatwoods salamanders (Bishop and Haas 2005). We also found that increased canopy cover was strongly associated with unsuitable wetlands (Table 8), and this is consistent with other studies that have found a negative association between increased canopy cover and suitability of ponds for larval amphibians (Skelly et al. 2002).

Although cover estimates of plant species are an obvious starting point for assessing habitat relationships of flatwoods salamanders, we also found evidence that the tolerance of plants to flooding conditions as defined by the NWI may also be useful in describing habitat. A preliminary analysis of grouping plants by both tolerance to flooding and structural attributes was not very informative because many combinations only contained a few species, so it was not substantially different from considering those species alone. Creative grouping of wetland plants based upon common ecological traits may be more informative than simply including plant species.

We also found that landscape structure was also an important predictor of habitat use in both logistic regression and discriminant function analyses. Including distance from streams improved prediction of habitat in regression analyses. Previous research has also shown that landscape

structure predicts presence of amphibians well (Van Buskirk 2005). Examining landscape pattern may be a useful starting point when habitat models are used to direct surveys and other management actions. Our original intent was to use landscape features related to military training and base operations to test for potential effects of these activities on larval presence in ponds. Other than roads and streams we were not able to include any other meaningful landscape features. Because the course and hydroperiods of many of the streams on the base have been largely modified due to base activities, the streams often interact with the wetlands in ways that are potentially harmful such as introducing silt, poor water quality, and predatory fish. Although our model predictions were improved by the inclusion of landscape features, we were not able to detect specific impacts related to base activities and these features. Because most of the flatwoods salamander habitat is found in close proximity to red-cockaded woodpecker colonies, the flatwoods salamanders benefit from conservation measures intended to preserve and protect woodpecker habitat, such as controlled burning and limitations on military training. Based on our many visits to the known and potential flatwoods salamander breeding ponds to survey and collect habitat data we did not observe obvious negative impacts of base activities.

The poor performance of habitat models in predicting occupancy of known breeding sites located elsewhere in the range of this species is striking (Figure 22). This is likely caused by differences in wetland vegetation and landscape structure among the sites in Florida and Georgia. These results are of general interest because many rare species persist on larger tracts of publicly managed lands scattered across their historic ranges. Although rare species may be well studied in one area, changes in habitat structure may cause models that are statistically robust where they are developed to perform poorly in new areas. A second consideration in applying habitat models is the status of the species in question. A species may decline for reasons that are not directly related to habitat structure (such as persistent drought), and if populations become restricted in their distribution or shift habitat associations as a result of other ecological factors such as disease, predation, and competition, then this may cause habitat models to perform poorly. Finally, the taxonomic status and biogeography of a species in question may also be important to consider. Pauly et al. (2007) recently proposed splitting *Ambystoma cingulatum* into two species with the Apalachicola River as a boundary between them. Unique habitat requirements for sister taxa or variation in habitats across ecoregional boundaries may make multiple habitat models for different taxa or regions more desirable.

Habitat modeling is a fundamental task related to the management of rare species. Models may be used to predict both the presence and the absence of a species, but validation is essential before using models to guide management activities, especially in areas that were not sampled for the development of the model. In addition to understanding what factors are related to the presence or absence of a rare species, it is important to understand the variation in habitat. While small numbers of potential sites may limit efforts to predict the presence or absence of a rare species, multivariate analyses need not be limited to sites where the presence of species has been documented. Thus, even in cases where locality data for a species are very sparse, it is still possible to determine if previously surveyed sites reflect the variation in habitat types in a landscape and the degree of similarity between known localities of a rare species. When confronted with the case of very rare species managers should remember that rarity affects the types of management questions that can be addressed by researchers, but rarity itself need not be viewed as an obstacle to further research that would better inform management actions. Rare

species are typically rare because they are found in low numbers or their distribution is restricted to only a few of the potentially suitable sites that exist in their range. In the case of the former, detectability may prove to be a significant issue affecting model performance when sites are considered to be unoccupied when they actually contain individuals of a species. The case of the latter could be more common for rare species. This would result in poor model performance when many suitable but unoccupied sites are similar to occupied sites. However, habitat modeling remains a valuable tool in spite of its challenges because it provides a means for quantifying habitat relationships and testing competing hypotheses about species distributions and management recommendations.

6. TASK 3: STOPPING RULES ANALYSIS

6.1 INTRODUCTION

As discussed in the introduction, the certainty with which one concludes that a pond is not inhabited by flatwoods salamanders is a function of several factors. Without some knowledge of that certainty, the choice of when to stop sampling a pond (either in terms of sampling effort per year or number of years of sampling) is largely made arbitrarily. This results in a classification of 'absence' of unknown certainty (or confidence) and creates a risk of either 1) undersampling and misclassification with environmental consequences or 2) oversampling with consequences of wasted effort that could better be applied elsewhere. In this task we developed a simulation model and performed an analysis in probability statistics to help determine how much sampling is necessary for a given pond to produce a specified level of confidence in declaring a pond is unoccupied.

An important aspect of sampling specifically to detect the presence of a rare species is that the capture of even a single specimen will halt the sampling and lead to the conclusion that they are present. Most of the effort in specifying design criteria in this study will go into the development of stopping criteria when a species has <u>not</u> been observed, while still avoiding an error of concluding that the rare species is not present when it actually is.

Reed (1996) and Kery (2002) apply a simple method for selecting the number of surveys to take prior to concluding absence based on probability of detection and a desired confidence level.

$$\text{Number of minimum visits} = \ln(\alpha \text{ level}) / \ln(1\text{-}p),$$

where α is level of acceptable Type I error (that is, concluding that a species is not present when it is) and p is the probability of detection (P[detection]). Table 13 shows the number of independent unsuccessful sampling events necessary to conclude that a species is not present at the stated levels of statistical confidence and P[detection]. Although the base equation is simple, the challenge we face is coming up with reasonable estimates of P[detection].

Table 13. Number of unsuccessful sampling events necessary to conclude with a specific confidence level that a species is absent

Statistical confidence	α level	Probability of detection		
		Low; p = 0.2	Medium; p = 0.5	High; p = 0.8
90%	0.10	11	4	2
95%	0.05	14	5	2
99%	0.01	21	7	3

The main objectives of this task were:

- To use results from field sampling in Task 1 to estimate salamander larvae detectability (i.e., P[detection]) at different larval densities, and

- To develop a simulation model to test the relative effectiveness of different sampling schemes with particular emphasis on rules for terminating sampling after repeated surveys without finding a target species (also referred to as non-detects).

The purpose of the 'stopping rules' model is to provide a tool that can be used to develop more efficient and higher confidence sampling designs. Field studies designed to address the same questions experimentally would take many years and huge amounts of effort to complete. As the project progressed, our approach to the stopping rules model evolved from a model that was to be primarily statistical in nature to one that was primarily simulation in nature. Simulation models similar to the one we developed have been used to test various strategies for controlling invasive species (Paice et al. 1988, Grevstad 2005).

6.2 METHODS

6.2.1 Detection Probability Analysis

The derivation of estimates of P[detection] and salamander abundance are closely related. P[detection] is dependent on surveyor sampling ability, the susceptibility of larvae to capture, and the density of larvae. In turn, larval abundance is a function of many things including habitat quality, metapopulation dynamics, and annual variation in environmental conditions.

During dipnet sampling for larval salamanders we routinely recorded the success (i.e., number of larvae captured) of individual netters for every 5 min of sampling. We used these data to derive estimates of P[detection] for individual ponds where flatwoods salamander larvae were found. The parameter of interest in the analysis of these data is the proportion of successful netters (i.e., those that detected at least one larva during a 5-min pass). We propose that the proportion of these 5-min individual passes with successful detections is equivalent to the P[detection], p, during a single 5 min sampling pass for that particular pond:

$$p_5 \approx \text{\# of passes with detects / total \# of passes}$$

For example, if three people dipnetted for 5 min each and only one was successful, the estimated of p_5 is 1 in 3 or 0.33. If the same three netters sampled for an additional 5 min each and two of the three captured larvae then the revised estimated of p_5 is 3 of 6 or 0.5.

6.2.2 Simulated Sampling

We used the R programming language to develop the sampling simulation program. R is an object-oriented language that is publicly available at no cost. The model framework is illustrated in Figure 29.

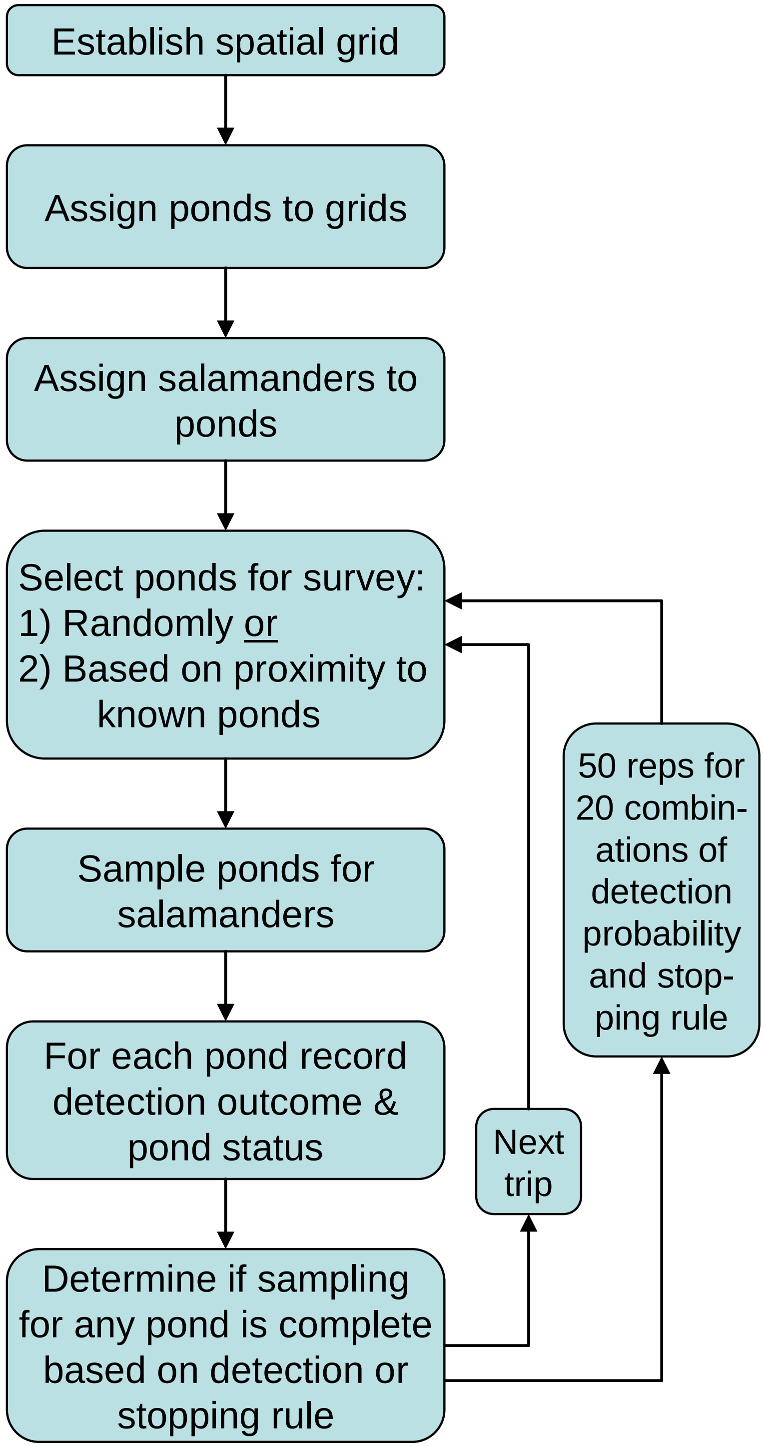


Fig. 29. Flowchart of simulated sampling model.

The first three steps in the model establish the ponds and salamander populations for sampling. We first created a rectangular grid of 90 by 120 cells. Second, the ponds were randomly assigned to roughly 4% or about 300 cells to create a 'pondscape'(Figure 30) with a number of ponds similar to the number of ponds in question at Fort Stewart. Next a percentage of the ponds was selected to be occupied by larval salamanders. For the baseline simulation, we chose roughly 10% of the ponds (i.e., about 33 ponds) to be occupied. The random selection of these occupied ponds was done such that their distribution was either randomly spaced or clustered. The number of clusters into which the ponds were distributed is selectable and was set at three for the baseline simulation.

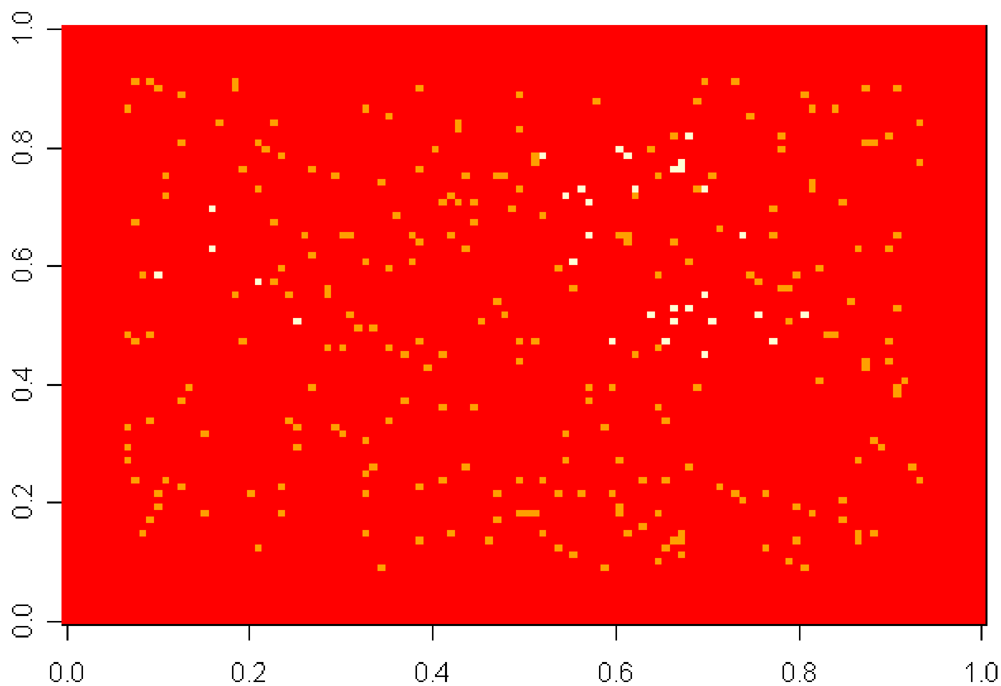


Fig. 30. Example pondscape used in simulated sampling model (120x90 cells). Orange cells contain unoccupied ponds and white cells contain ponds with larval salamander present. This example shows a high level of clustering of occupied ponds into three main groups of ponds.

The second set of steps in the model samples ponds for occupancy based on various sampling rules. At the beginning of each pass a list of ponds to be sampled was generated. These lists were sampled in one of two ways, either completely randomly or adaptively. Random sampling generated a list randomly from the ponds that were still available for sampling, i.e., those in which larvae were not detected previously and those that had not been removed from the list because the number of consecutive non-detects had not yet met a stopping rule. Adaptive sampling used information collected on ponds where larvae were detected during the first pass of randomly selected sites to select ponds in subsequent sampling passes. In our case, we used physical proximity to detected ponds as a predictor of likely occupancy. Ponds that were closest to previously detected ponds were chosen for sampling in the next round.

Proximity to detected ponds was recalculated after each sampling pass as the number of detected ponds changed. The number of ponds sampled per pass and the number of passes is selectable as an input parameter. The baseline simulation consisted of 10 passes of 50 ponds each pass. With Fort Stewart as a basis for these simulations we can consider each pass as a season of sampling. However, it is also appropriate from a modeling perspective to consider these passes to be completed several per season or even all in one season depending on the goals and assumptions of the model scenario being simulated.

When a pond is selected for sampling, a random number is generated and compared to the probability of detection which is the same for all ponds. If the random number is less than the detection probability, the pond is considered occupied. Once all the ponds selected for a pass are sampled, results are recorded and evaluated. Ponds that have been sampled unsuccessfully for a number of times equal to the current stopping rule are excluded from future sampling. For very low stopping rule values it is possible that all ponds are either detected or reach the stopping rule, in which case those that had reached the stopping rule are returned to the list of available ponds. In this way, the total number of ponds sampled remains constant among the different sampling scenarios.

For each pond in the simulation, the model records how many times it was sampled and the result of each sample. For the entire population of ponds, we tracked the number of surveys of both occupied and unoccupied ponds and the number of successful and unsuccessful surveys of occupied ponds.

We simulated 200 combinations of different environmental and sampling options (Table 14). Environmental options included number and distribution of occupied ponds and probability of detection. Sampling options included different stopping rules, sampling mode (random or adaptive), and number of passes and trips. Each scenario was simulated 50 times and average values stored for analysis.

The baseline simulation for both modes of sampling, random or adaptive, consists of:

- 20 combinations of four levels of detection and five levels of stopping rule,
- highly clustered distribution of occupied ponds (3 clusters),
- low density of occupied ponds (~10% of all ponds), and
- 10 trips of 50 ponds sampled per trip.

In other simulations we tested a different combination of trips and ponds per trip, a higher density of occupied ponds, and different distributions of occupied ponds (less clustered and no clusters).

The R code for the program used in the simulations is included in Appendix A.5 and is also available from the authors.

Table 14. Factors involved in generating different sampling schemes for comparison with the simulated sampling model

Simulated Sampling Factors	Levels
Probability of larvae detection (usually related to density) • 0.2, 0.4 , 0.6, 0.8	4
Stopping rule (number of consecutive non-detects before giving up) • 1, 2, 3, 4, 5	5
Distribution of occupied ponds • Random (i.e., no clusters) • Few clusters of many ponds each • Many clusters of few ponds each	3
Number of occupied ponds • Low and high (10% and 25% of total ponds)	2
Sampling approach • Random (ponds selected at random) • Adaptive (ponds selected based on factor correlated with presence	2
Effort distribution (ponds per trip) • 50 ponds each for 10 sampling periods • 100 ponds each for 5 sampling periods	2

6.3 RESULTS AND ACCOMPLISHMENTS

6.3.1 Capture Probability Analysis

Our dipnet sampling at Fort Stewart and the three Florida locations resulted in 13 instances where we captured flatwoods salamander larvae and were able to estimate P[detection] based on the proportion of successful 5-min periods (Table 15). Capture rates varied from 0.014 to 0.66 larvae per minute. P[detection] ranged from 0.07 to 1.0. The relationship between capture rate and capture probability is illustrated in Figure 31.

Using some basic probability statistics, we were able to convert 5-min P[detection] to probabilities for sampling periods of other durations. For example, if p_5 is the P[detection] for 5 min of effort then the probability of <u>not</u> capturing a larva in 5 min is

$$1 - p_5,$$

and the probability of <u>not</u> capturing a larva in 10 min (i.e., two 5-min passes) is

$$(1 - p_5) \cdot (1 - p_5) \text{ or } (1 - p_5)^2$$

Table 15. Larval flatwoods salamander capture success for one pond at Fort Stewart (sampled as two distinct sections - main body [m] and peripheral logging tracks [t]), five ponds at St Marks NMR, three ponds at Apalachicola NF, and one pond at Eglin AFB. Number of larvae captured by individual netters for each 5-min pass is included except for A10.2-02m Apr 05 sample during which captures for individual netters was not recorded

Pond	A10.2-02m	A10.2-02t	smnwr 0111	wakdc 0009	anf 28.002	anf 100.11	anf 77.011	smnwr 0110	smnwr 0108	smnwr 0115	A10.2-02t	A10.2-02m	eg16
Mo Yr	Apr 05	May 05	Feb 07	Feb 07	Feb 07	Feb 07	Feb 07	Feb 07	Feb 07	Feb 07	Feb 08	Feb 08	Mar 07
Netters	3	2	6	6	6	6	6	7	7	7	3	3	4
Larvae caps	14	11	13	28	29	2	18	46	30	2	2	7	6
Total min	223	70	35	60	60	60	30	70	70	140	75	60	36
Capture/min	0.063	0.157	0.371	0.467	0.483	0.033	0.600	0.657	0.429	0.014	0.027	0.117	0.167
Detects	11.5	7	6	10	9	2	5	12	11	2	1	5	4
Non-detects	32.5	7	0	2	3	10	1	0	3	26	14	7	4
Prop. detects	0.26	0.50	1.00	0.83	0.75	0.17	0.83	1.00	0.79	0.07	0.07	0.42	0.50
Number of larvae captured by individual netters during consecutive 5 minute passes													
1st	0	1,0	2,4,1,1,3,1	1,0,2,4,0,9	4,6,3,2,1,0	1,1,0,0,0,0	2,9,0,1,2,4	5,1,2,5,5,2,4	3,2,2,1,1,2,0	0,0,0,0,0,0,0	0,2,0	0,0,0	0,0,0,1
2nd	0	0,1		4,1,3,1,1,2	6,3,0,0,3,1	0,0,0,0,0,0		2,1,1,10,3,2,2	7,3,0,3,0,2,4	1,1,0,0,0,0,0	0,0,0	0,1,0	1,1,0,3
3rd	0	1,2								0,0,0,0,0,0,0	0,0,0	1,0,1	
4th	2	0,0								0,0,0,0,0,0,0	0,0,0	0,3,1	
5th	1	0,0									0,0,0		
6th	2	2,0											
7th	0	2,2											
8th	2												
9th	1												
10th	0												
11th	1												
12th	2												
13th	2												
14th	1												
15th	0												

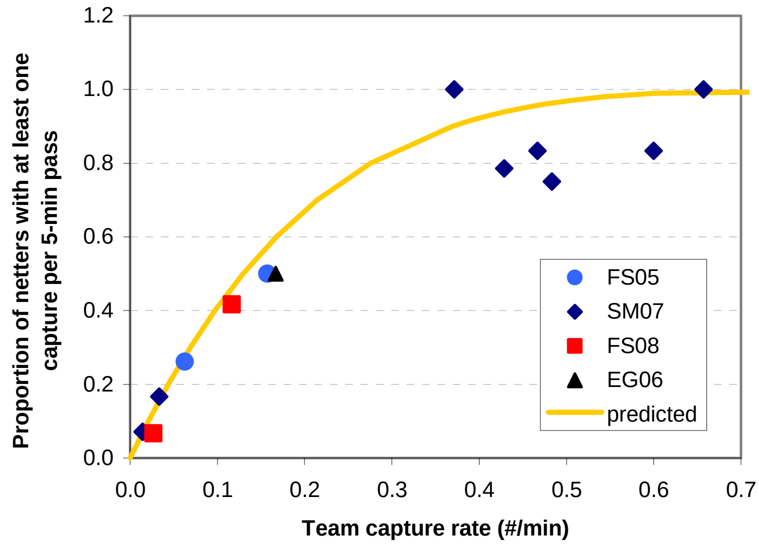


Fig. 31. Relationship between team capture rate and P[capture] determined both experimentally and theoretically.

It then follows that the probability of detecting a larva in 10 min is

$$1-(1 - p_5)^2$$

This same reasoning can be used to calculate the P[detection] (or non-detection) for any amount of sampling time greater than 5 min. For example, the probability of not capturing a single larva in 60 min when p=0.3 is

$$(1 – 0.3)^{12} = 0.014$$

Similarly, one can calculate the P[detection] for a 1-min pass from a 5-min probability as

$$1-(1-p_5)^{1/5}$$

For example, the probability of detecting at least one larva in 1 min, p_1, when p_5=0.3 is

$$1-(1-.3)^{1/5} = 0.069$$

We propose that this estimate of the P[detection] for a 1-min pass is equivalent to the overall per minute capture rate (line 6 in Table 15) when larvae are randomly distributed throughout the specific area being sampled and all netters have equal probability of encountering larvae. Given those assumptions, a second way to estimate a 5-min probability of detection is to use the per minute capture rate as an estimate of the P[detection] during a 1-min pass and from that calculate the 5-min (or other time period) probability. For example, a capture rate of 0.2 larvae per minute corresponds to a 5 min P[detection] of

$$1-(1-.2)^5 = 0.67$$

Figure 31 includes the predicted or theoretical 5-min P[detection] as a function of per-minute capture rate for comparison to the observed values. Figure 32 compares directly the observed P[detection] and the theoretical probability for the corresponding capture rate.

The above approach can be applied to any length of sampling time either by an individual or group as shown in Figure 33.

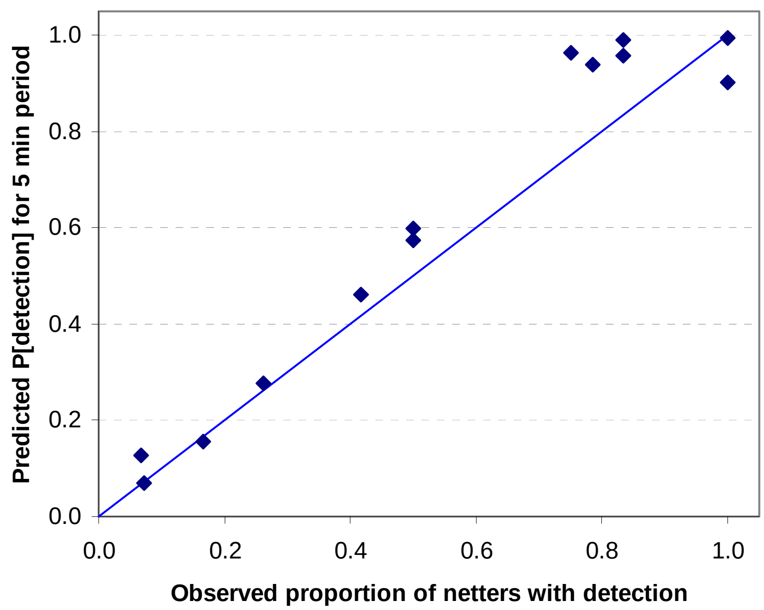


Fig. 32. Plot of observed versus hypothetical 5-min detection probability at specific capture rates. Line is 1:1 relationship.

6.3.2 Simulated Sampling

The goal of the sampling simulations was to compare the ability of various sampling schemes (i.e., different combinations of sampling mode and stopping rules and allocation of effort) to successfully sample occupied ponds under different environmental conditions (i.e., number and distribution of occupied ponds and larvae detectability). Each panel in Figures 34 and 35 compares 20 different combinations of probability of detection (0.2, 0.4, 0.6, 0.8) and stopping rules (1, 2, 3, 4, 5) for various sampling scenarios. The figures show the proportion of the 30 occupied ponds that were either 1) sampled and detected, 2) sampled but undetected, and 3) never sampled. Depending on the question of interest there may be other metrics that are of interest, such as the amount of effort expended at unoccupied ponds.

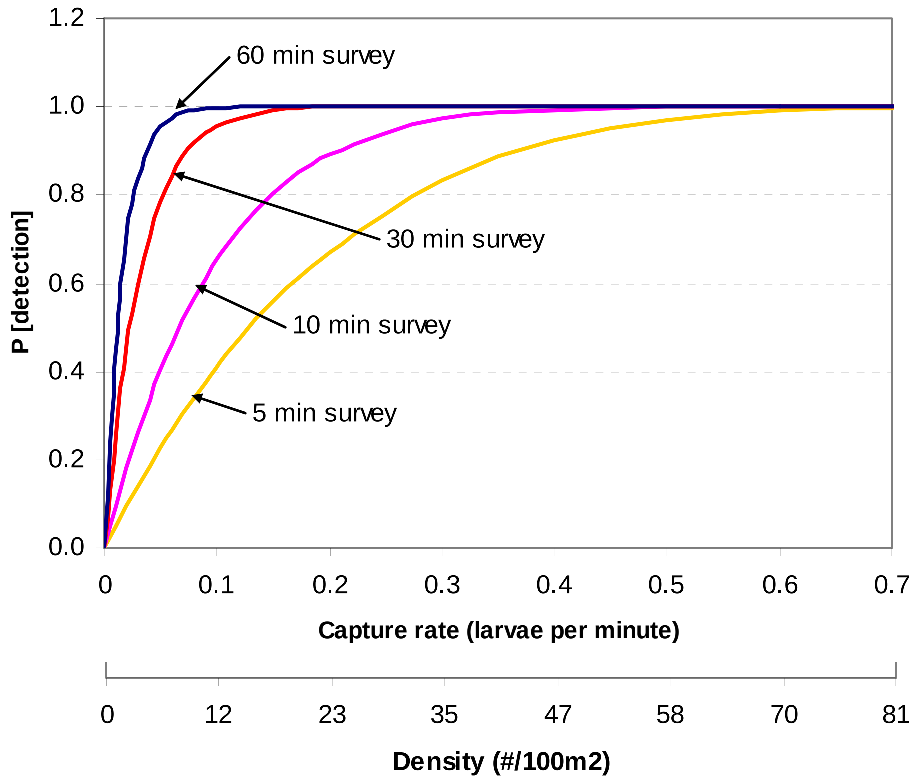


Fig. 33. Theoretical relationship between per minute larval capture rate (and density) and probability of detection for various length surveys. Density axis based on relationship between capture rate and density shown in Figure 20.

Panels A and B of Figures 34 and 35 show the results for the baseline conditions for the two sampling modes, random and adaptive. (Note: Panels A and B are repeated in Figure 35 for ease of comparison with the other simulations.) With random sampling of the baseline conditions (Figure 34A), we see that at the lowest detection (left group of frequency bars) there is little difference in the number of detected ponds for the different stopping rules and that stopping rules of 1 or 2 result in the fewest number of ponds not sampled. As detection level increases, we see an increase in the number of detected ponds across all stopping rules as expected, but more importantly, a difference in number of detected ponds among stopping rule values in favor of 1 and 2.

When using adaptive sampling under baseline conditions (Figure 34B), we found that high stopping rules generally resulted in greater detection across the different levels of detectability. At low detectability, the maximum stopping rule of 5 times is best, but as detectability increases a stopping rule of 4 and eventually 3 are equally productive. In addition, the adaptive sampling resulted in more detections than random sampling at every combination of detectability and stopping rule.

The baseline condition included a relatively high level of clustering of occupied ponds (see Figure 30). We also simulated sampling from a loosely clustered distribution of ponds (about 17 clusters of 2 ponds each; Figure 34, panels C and D) and from a random distribution with no

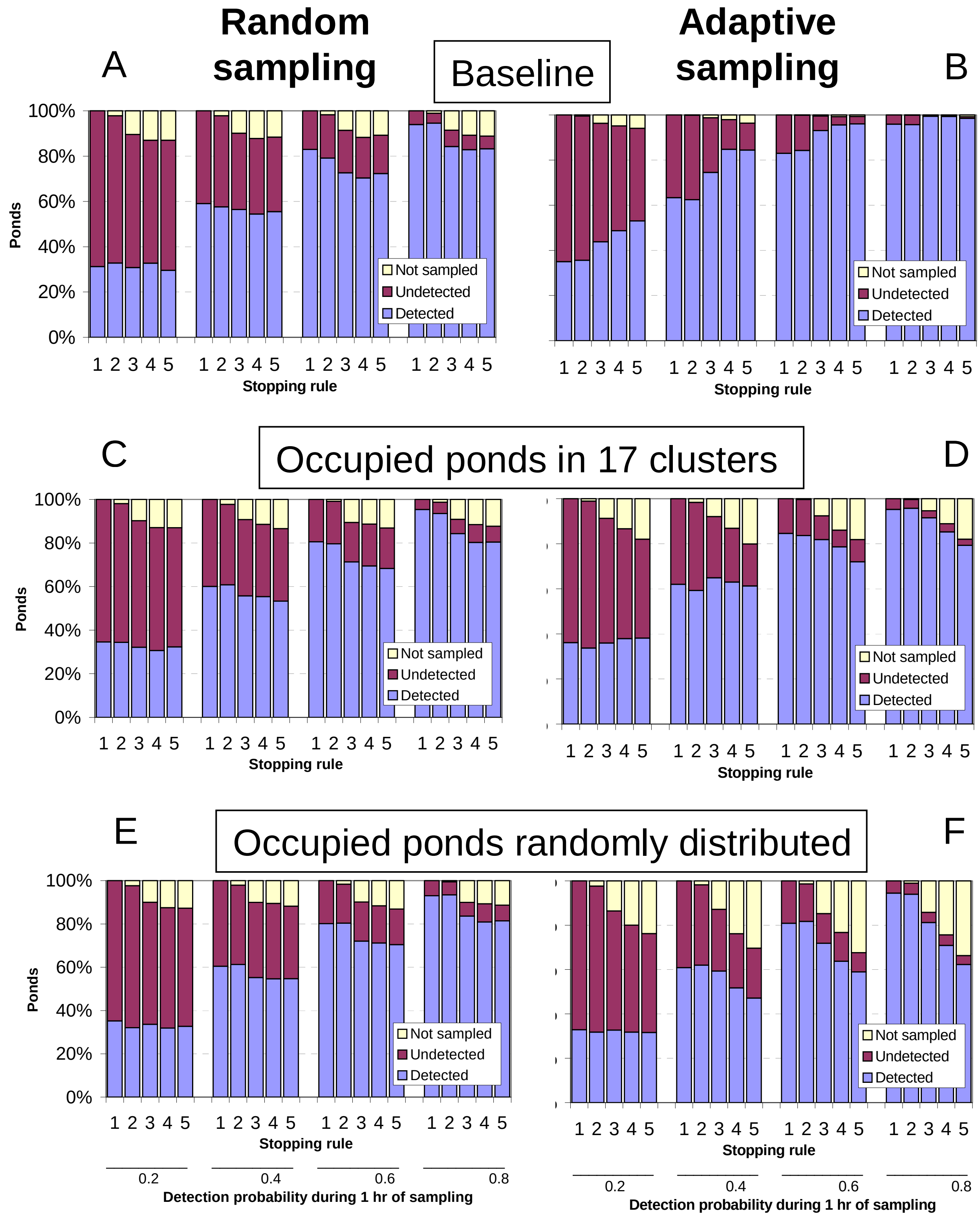


Fig. 34. Simulated sampling results for occupied ponds only; simulations included various sampling schemes and different distributions of occupied ponds.

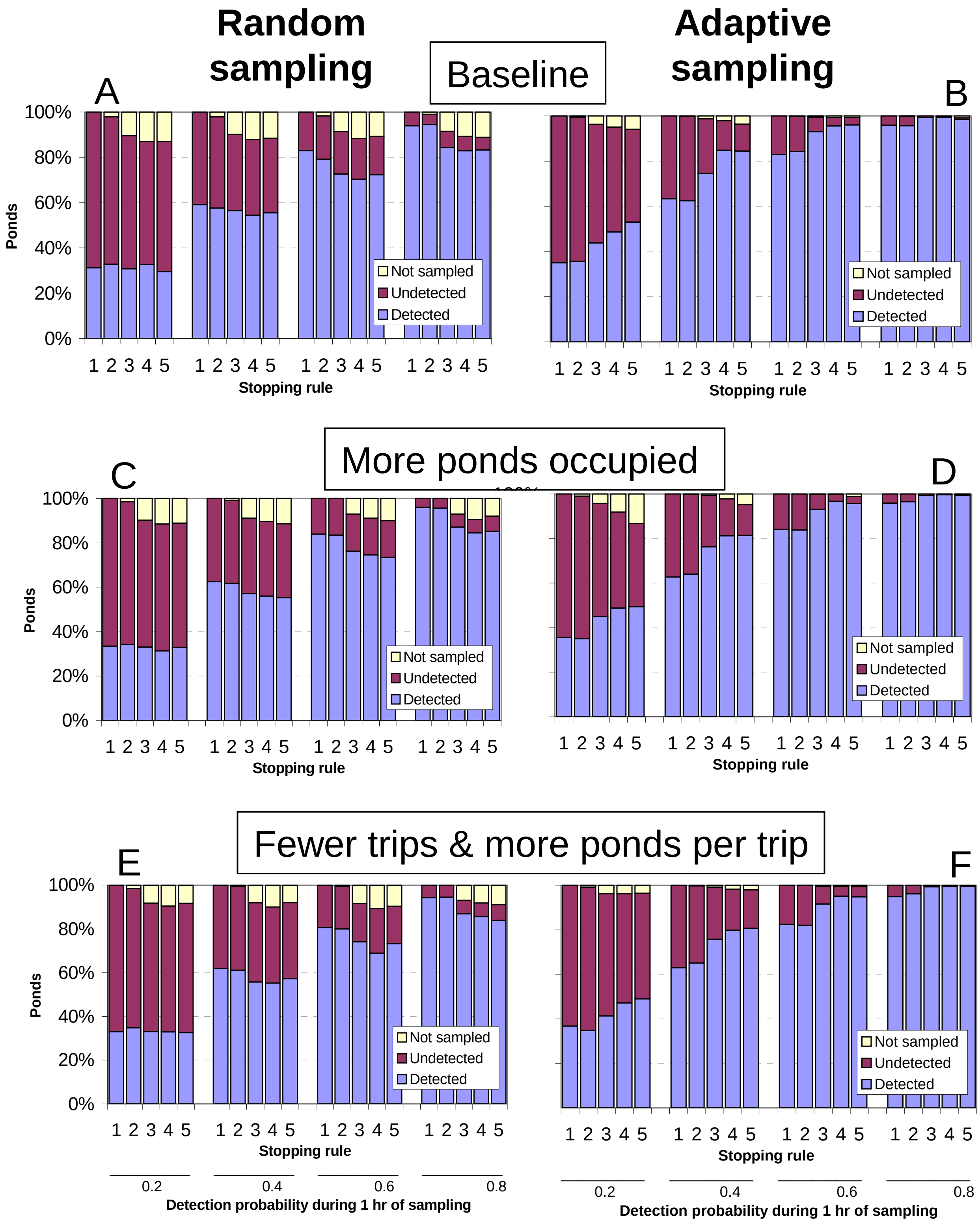


Fig. 35. Simulated sampling results for occupied ponds only; simulations included various sampling schemes and differences in number of occupied ponds and allocation of sampling trips.

clustering (i.e., occupancy was totally randomly assigned; Figure 34E and F). For the random sampling alternative (Figure 34, panels A, C, and E), there is virtually no difference in the sampling success for the different detectability-stopping rule combinations among the three levels of clustering. However, for adaptive sampling, as clustering becomes less pronounced the advantage of high value stopping rules reverses and a stopping rule of 1 or 2 is most successful (Figure 34, panels B, D, and F).

We increased the number of occupied ponds by a factor of 2.5 to see if that factor had an effect on the relative performance of the two sampling methods and found virtually no difference in incidence of detection for any combination of sampling designs (Figure 35, panels C and D). We also changed the distribution of effort from 10 trips of 50 ponds sampled each trip to 5 trips and 100 ponds per trip and found no effect on sampling success relative to the baseline (Figure 35, panels E and F).

6.4 DISCUSSION

Detection Probabilities, Capture Rates, and Certainty of Absence

The need to know the probability of detection to be able to estimate a level of certainty in declaring absence after repeated non-detects presents an interesting dilemma. Since probability of detection for a specific pond is (at least in our case) a function of density, the only way to know the probability of detection for a pond is to do testing that involves collecting at least one individual. Of course, if an individual is detected, the need to declare absence is no longer an issue. Therefore, in unknown ponds we either need to 1) be able to estimate probability of detection based on other information (such as P[detection] in a similar pond) or 2) select a minimum density above which one is interested in knowing with confidence whether a pond is occupied. For example, resource managers might decide that conservation efforts are best spent on ponds with healthy populations and ponds with little to no production would be assigned a low priority for management. Once we know the relationship among probability of detection, capture rate, and density, we can easily calculate the probability that a particular number of consecutive non-detects could occur if the population was indeed present at some specified density.

We derived two ways to estimate probability of detection from field survey data. One method uses the success rate of individual netters during regular sample intervals, for example, the proportion of successful 5-min sampling periods for all netters. The other method uses the average per minute capture rate for the entire team to predict detection probability for longer time periods. There are other ways to derive P[detection] that require more manipulation of the organisms or more sampling effort. For example, repeated sampling of the same area (e.g., pond or portion of a pond or other habitat) that results sometimes in detection and sometimes not can be used to estimate detection probability. Such sampling has to occur over a short period during which there is no immigration, emigration, or mortality, and the samples can not be either 100% successful or 100% unsuccessful. Another often used method is to place a few marked individuals into an enclosed area and after repeated surveys use rate of capture of some or all marked individuals to calculate probability of detection. We were not able to use these other

methods due to the rarity of flatwoods salamanders at Fort Stewart and the bureaucratic hurdles involved in gaining permission to tag or mark a federally listed species.

That we were able to estimate probability of detection is extremely important in being able to estimate how confident we are in declaring absence after repeated unsuccessful surveys, especially when combined with information on the relationship between capture rate and density. As discussed earlier, for any given probability of detection, p, for a single sampling trip, the probability of not detecting a larva in x trips is

$$(1 - p)^x$$

A plot of this relationship for 1 to 20 consecutive non-detects illustrates that at low detectability the number of consecutive non-detects necessary to become confident that larvae are truly absent (say below a probability of 0.1) can be very high (Figure 36). For example, at a P[detection] of 0.4 nearly 5 consecutive non-detects are necessary to exceed the 0.9 probability that 5 consecutive non-detects could occur by chance. Similarly, at a p[detection] of 0.1, about 20 consecutive non-detects are necessary to reach this 90% level of confidence.

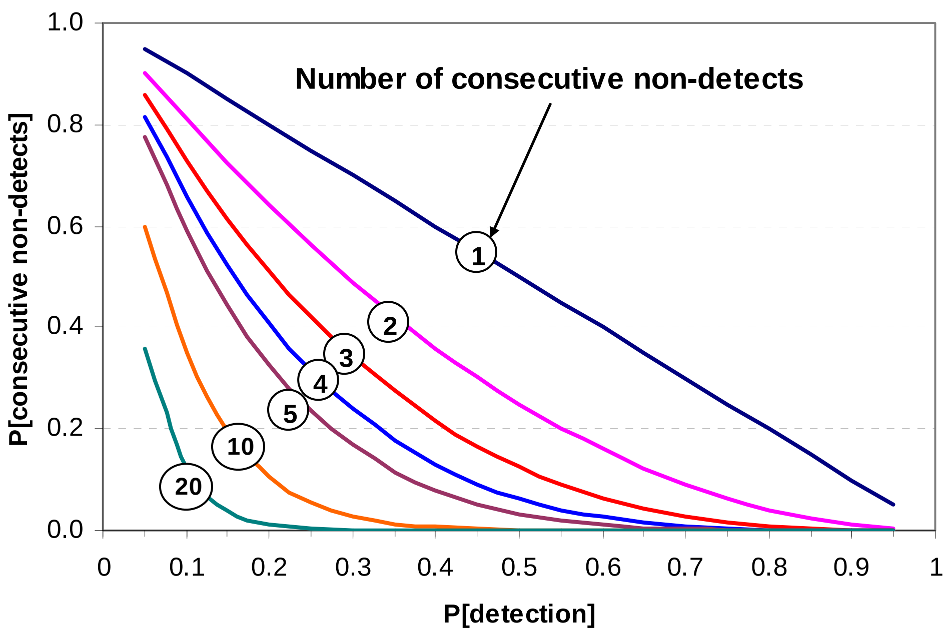


Fig. 36. Relationship between probability of detection and probability of 1, 2, 3, 4, 5, 10, and 20 consecutive non-detects if present.

For comparison, P[detection] for 5 min sampling periods ranged from 0.07-0.5 for different dates at the Fort Stewart occupied pond. These rates translate to 60-minute P[detection] of 0.58 to >0.99. At St Marks P[detection] ranged from 0.07-1.0 for 5 min of sampling or 0.58 to 1.0 for 60 min. At Apalachicola P[detection] ranged from 0.17-0.83 for 5 min of sampling or 0.89 to >0.99 for 60 min. The wide range of P[detection] we observed among ponds or among dates at the same pond could be a result of differences in larval density among ponds (or in different areas

within the same pond) or differences in density through time as a result of mortality or emigration.

The number of consecutive non-detects (x) at a particular pond needed to be certain with an established level of confidence that the pond is unoccupied can be calculated as follows:

$$x = \ln(\alpha) / \ln(1 - p)$$

where α is the confidence level (i.e., α=0.05 equates to 95% confidence) and p is P[detection]. Note that this calculation presumes that the each trip (or sampling event) would represent the same duration of sampling as that upon which the detection probability is based. The number of consecutive trips necessary to achieve 90, 95, and 99% confidence of absence for a range of P[detection] is illustrated in Figure 37. For example, using the lowest probability of detection calculated from the SERDP study (0.58 for an hour of sampling), it would require 4 consecutive 1-hr surveys without finding a larva to be 95% confident that a pond is unoccupied.

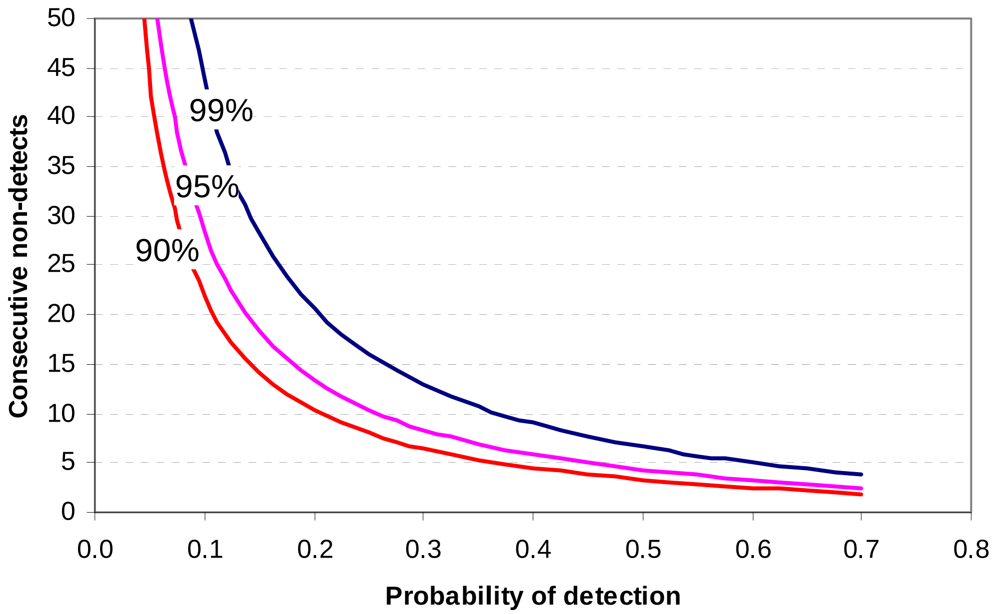


Fig. 37. Relationship between probability of detection and the number of consecutive non-detects needed to achieve a 90, 95, and 99% level of confidence that larva are actually absent.

Simulated Sampling

One goal of the simulated sampling exercise was to evaluate the relative effectiveness of random versus adaptive sampling. We found that neither method was better than the other in all circumstances. In general, when occupied ponds were distributed in clusters adaptive sampling produced more detections than random sampling. When occupied ponds were randomly distributed across the pondscape, random sampling was slightly better. Note that when sampling

effort is high, adaptive sampling with a stopping rule of one is practically the same as random sampling since the low stopping rule results in all of the ponds being sample.

In the case presented here, the adaptive sampling was successful because occupied ponds were clustered and the adaptive sampling was based on proximity of ponds to previously detected ponds. Other factors can be used as a basis for adaptive sampling as long as there is a known correlation between that factor and salamander occupancy. Other possible factors that might serve as a basis for adaptive sampling are: pond hydroperiod, presence or absence of some specific pond vegetation, absence of predator species, or presence of an indicator species such as another amphibian with similar life history.

Another goal of the simulation exercise was to assess how soon to stop sampling after repeated non-detects and move on to other ponds. When selecting ponds randomly, there were no circumstances when a stopping rule of more than two was advantageous. Under adaptive sampling the effectiveness of using stopping rules depended on the circumstance. When the ponds were highly clustered, higher stopping rules were most effective, especially when the P[detection] was low; as P[detection] increased, the difference between the range of stopping rules decreased. In the two examples with reduced clustering and no clustering, high stopping rules were usually counter-productive because too much time was spent in unoccupied ponds (Figure 34). These results suggest that adaptive sampling should only be employed when there is a known correlation between the basis for the adaptive sampling and salamander presence.

Lastly, we found virtually no difference in these results between simulations with pond occupancy rates of 10% and 25% of the total ponds. Similarly, a different distribution of trips and number of ponds per trip made no difference in the relative effectiveness of the two modes of sampling and the different stopping rules.

The simulations we performed assumed no prior knowledge of occupancy in the ponds being sampled. After the first round the adaptive approach did use detection information to adjust sampling. In reality, for nearly all of the sites where flatwoods salamanders exist there is some prior knowledge of occupancy. The simulation strategy or model code could easily be adapted to deal with this if desired. For example, if there is no basis for adaptive sampling one could simply leave the already known ponds out of the simulations and just work with the remaining. Alternatively, the presence of known ponds could be treated as the results of the first random pass and the model could proceed from that point. The general results at the end would likely remain the same.

7. PROJECT SUMMARY AND CONCLUSIONS

7.1 RESULTS SUMMARY

The primary objective of this study was to maximize field sampling efficiency of flatwoods salamanders and minimize the uncertainty associated with declaring absence after repeated non-detects. Our approach was to learn more about sampling method efficiency, the relationship between pond habitat and larvae residency, and the effect of sample design on sampling success.

Field Surveys

We surveyed salamander larvae and pond habitat at Fort Stewart for three years and at several locations in Florida. Our studies on method effectiveness demonstrated that the most time efficient and cost effective sample method is dipnetting. Larvae were detected at one pond at Fort Stewart in three of four years of the study, but not at 59 others that were sampled. These detections represent the only observations of successful breeding in a natural wetland in Georgia since 1999. Our inability to find larvae in more than one pond at Fort Stewart confirms what appears, based on historic capture records, to be a significant decline in flatwoods salamander abundance at Fort Stewart. To better predict annual variation in pond residency, we developed a model that uses rainfall data and likely growth rates to predict hatching dates and period of pond residency. With two years of data that likely represent the extremes of pond residency in Georgia we found that date of hatching can occur as early as the first week of January and as late as the first week of March and, that in some years, larvae may be found in the pond as late as the end of May. We recommend that the most opportune time of year to sample flatwoods salamander larvae is during the second and third months after a pond fills to at least half, typically sometime within February to April. Using removal study data from temporary enclosures at St Marks NWR we derived a relationship between larval density and capture rate that allows future estimates of larval density based on observed capture rates. The proportion of total larvae present that were captured during a pass averaged about 40% (range of 22-65%) and was not dependent on larval density.

Habitat Modeling

We used statistical models to predict larval occupancy of wetlands and to quantify the similarity among wetlands based upon habitat data from pond-specific vegetation and landscape data. The presence of flatwoods salamanders was positively associated with a native iris species and the presence of facultative and obligate wetland plants, and increased canopy cover was strongly associated with unsuitable wetlands. Landscape structure was also an important predictor of habitat use. For example, including distance from streams improved prediction of habitat in regression analyses, and discriminant function analyses with landscape data had a higher rate of correctly classifying known breeding sites as being occupied. Models developed for Fort Stewart populations did a poor job of predicting presence for sites at St Marks NWR and Apalachicola NF. Results from hierarchical clustering indicate that this may be due to differences in wetland vegetation and landscape structure among the sites in Florida and Georgia. Developing habitat models for rare or declining species is particularly challenging because a species may decline for

reasons that are not directly related to habitat structure, and if populations become restricted in their distribution or shift habitat associations as a result of other ecological factors such as disease, predation, and competition, then this may cause habitat models to perform poorly. Amphibians are also challenging subjects for habitat modeling because their occupancy in ponds is highly variable through time. Because the habitat modeling did not identify the habitat of the historically occupied ponds at Fort Stewart as particularly unique, we believe that either 1) there are many ponds at Fort Stewart that are capable of supporting flatwoods salamander breeding or 2) the features of the habitat that we used as input variables to the models do not include all the important features.

Sample Design

Estimating the probability of detection is crucial to being able to calculate how many unsuccessful surveys are necessary to conclude that a site is unoccupied with a known level of confidence (e.g., 95%). We developed two methods using the larval survey data to estimate probability of detection, which is a key component for calculating sampling effort needed to be confident that an undetected pond is actually unoccupied. The first method uses detection success rate for 5-min survey intervals and the second uses per-minute capture rate. With either method we can relate probability of detection to capture rate and, based on our enclosure study results, also to larval density. These relationships allow us to base calculations of number of necessary surveys on an expected larval density instead of a detection probability, a concept that for most biologists has less meaning and utility than population density. Therefore, when an estimate of detection probability for a pond is unavailable or unattainable, biologists have two options if they want to determine how many surveys are necessary. They can either 1) select a minimum density above which they want to be reasonably sure larvae do not exist and calculate the required number of non-detects necessary to reach that conclusion, or 2) select a level of confidence with which they are comfortable and, knowing that their sampling effort is limited to a set number of surveys, they can calculate the density above which they are reasonably certain that larvae do not exist.

We developed a computer model to simulate periodic sampling of ponds for flatwoods salamander larvae. The model was used to determine the effectiveness of random versus adaptive sampling and the effect of stopping rules on survey success. We found that neither sampling mode was better than the other in all circumstances. In general, when occupied ponds were distributed in clusters, adaptive sampling based on pond proximity produced more detections than random sampling. In fact, if surveyors are able to identify any factor that is correlated with salamander presence, then adaptive sampling with that factor as a basis will produce the most efficient sampling design. When occupied ponds were randomly distributed across the pondscape, random sampling was slightly better. With regards to stopping rules, when adaptive sampling is justified, stopping rules of four or five produce more detections, but when surveyors have no basis for adaptive sampling, random sampling with a stopping rule of one or two trips is better or equal to longer stopping rules. The model was coded in the R, a programming language which is widely used and available at no cost.

7.2 IMPACT OF LIMITED SAMPLING SUCCESS

This study was originally based on the expectation of finding flatwoods salamander larvae in several ponds at Fort Stewart. Under this expectation, method comparison results would have been averaged and compared across several ponds allowing us to make conclusions about the transferability of these results to other ponds at Fort Stewart and elsewhere. Unfortunately, we only found larvae at one pond at Fort Stewart. Therefore, our analysis of method effectiveness, hatching dates, larvae size, and capture rates for Fort Stewart are based on a single pond. With regard to the relative effectiveness of the different collection methods we believe that the data collected are representative of other ponds across Fort Stewart. Within pond habitat was similar across the base and we expect that dipnetting would prove to be the most effective method if successfully tested at other Fort Stewart ponds. For other aspects of the study we were able to substitute or supplement Fort Stewart data with results from complimentary studies we performed at three sites in Florida. By using the Florida data in place of Fort Stewart data we assume that the sites are similar enough that the conclusions would be the same. For example, we assume that the relationship we derived between capture rate and larval density based on removal studies at St Marks is representative of sites elsewhere. Because we think that flatwoods salamander larval behavior with regard to how it normally hides in vegetative cover and how it might move when disturbed are fairly consistent range-wide, and thus the catch relationships we derived should also be consistent range-wide. For estimation of detection probabilities, we required survey success data from ponds of different larval densities and detection rates. It would have been difficult to accomplish this with the single pond at Fort Stewart and may have caused undue stress to larvae as a result of repeated captures. Fortunately, we were able to combine data from all four sites and developed a relationship that appears applicable for all sites (see Figure 31). Had we had more Fort Stewart ponds with capture success and detection data, it would have been useful to look for correlations between habitat variables and capture or detection rates.

From the habitat modeling we had hoped to get information on the likelihood of a pond to support flatwoods salamander reproduction based on its habitat quality. Multivariate analyses of habitat relationships are informative even in the absence of much data on where salamanders actually occur. In lieu of recent results of salamander occupancy at Fort Stewart we relied on historic data from the past 15 years of sampling with the assumption that present habitat quality in these ponds is similar to what it was when larvae were last found there. Although additional data is always more desirable, results from our cluster analyses clearly show variation in habitat across the range of the species at the scale of individual ponds and surrounding landscapes. These findings are critical in understanding the appropriateness of applying habitat models to different areas. Had we found larval salamanders in several ponds we also hoped to explore a possible relationship between habitat quality and larval abundance. We were not able to derive these relationships and so relied on a broader set of assumptions for our stopping rules model. For example, since we do not know to what degree habitat quality affects presence and abundance, we ran the sampling model under the assumption that there was no relationship between habitat and abundance.

We do not believe that any of the information deficiencies described above prevented us from developing a stopping rules model that would provide meaningful and useful insight into effective sampling designs. We originally had intended to produce a simulation model that was

spatially explicit based on Fort Stewart pond distributions and would rely on habitat and abundance relationships developed from earlier tasks. Instead we developed a more generic model, but one that was still on scale with Fort Stewart with respect to total number of ponds and proportion of ponds occupied. In hindsight, we feel that the non-specific nature of the sampling model as developed makes it easier to detect and understand the underlying behavior of the model and its output. We believe the results are widely applicable across the range of flatwoods salamanders and were not compromised by the lack of additional Fort Stewart data.

7.3 TRANSITION PLAN

Workshops Hosted

To ensure distribution of the results and recommendations of this study, we conducted a workshop with regional flatwoods salamander biologists during the first year of the project to gather feedback on our study objectives and design, and a second workshop at the end of the project to discuss results. The final workshop was attended by 18 biologists including representatives from USFWS, US Forest Service, Fort Stewart Fish and Wildlife Branch Office, Florida Fish and Wildlife Conservation Commission, Georgia Department of Natural Resources, the Jones Ecological Research Center at Ichauway, the University of Georgia, and several private consultants that do contract work for these agencies. These individuals are responsible for the management and assessment of flatwoods salamanders across their entire range from western Florida to South Carolina. We presented the results of the project and discussed ways to make the information more useful for field biologists and managers. Attendees reported on the status of flatwoods salamanders at the sites they manage and sample. Florida sites seem to be maintaining status quo, but other than the one site at Fort Stewart, no breeding has been documented at any other location in Georgia or South Carolina. At the end of the workshop we had attendees complete a questionnaire to provide feedback to us on the usefulness of our project results and on flatwoods salamander conservation challenges in general (Table 16).

Other

In February 2008 Dr. Bevelhimer met with the chief of the Fort Stewart Fish and Wildlife Branch Office (Tim Beaty) and the manager of their endangered species program (Larry Carlile) to discuss results of the projects and specific recommendations for Fort Stewart with regards to flatwoods salamander conservation efforts. The information was well-received and they expressed an interest in applying the approach for other rare species on the base. During this trip, we also accompanied the biologist who was recently hired at Fort Stewart to manage flatwoods salamander monitoring into the field to sample several ponds and discuss sampling techniques and design.

Technical publications are listed in Appendix B.1.

Table 16. Questionnaire results from 2008 flatwoods salamander workshop

QUESTION	SUMMARY OF ANSWERS
What is your affiliation?	Federal 3 State 3 University 1 Private/Consultant 3
Where do you work with flatwoods salamander populations?	Florida 6 Georgia 7 South Carolina 2
Possible reasons for apparent continued decline of species? (rank top five reasons with 1 being highest)	Average Rank 1. Habitat 1.1 2. Fragmentation 2.4 3. Drought 2.8 4. Water Quality 3.4 5. Disease 4.8
What are most important mitigation measures that should be continued, enhanced, or employed?	• Protection, conservation, restoration and management of both upland and breeding habitat. • Fire – The preclusion of fire has and will continue to have negative impacts on flatwoods salamander habitat. Need burning of woodlands surrounding breeding ponds. Need to properly manage habitat with growing season fire or during drought periods so ponds will burn, as well as adjacent uplands. • Where fire does not do so, reduce/remove shrubby vegetation with cut-stump herbicide treatment. • Maintain connections among proximate wetlands. • Establishment of protocols for reintroduction should be a priority. Mitigation will not have any real benefit in many cases unless a new population can be established.
What are the greatest bureaucratic obstacles hindering flatwoods salamander conservation?	• Need a better policy for dealing with surveys, protection and management of flatwoods salamanders on private lands. There needs to be a better balance struck between landowner rights and the ability of agencies to protect the species on private lands. • Lack of funding and personnel to conduct surveys and in some cases properly manage habitat (e.g. for burning). Funding is specifically need to support long-term studies. • Habitat destruction, setting aside and properly managing existing habitat. • Risk of getting no data during graduate student research if there is a drought. • Difficulty in getting burn permits during a summer drought. • Need new programs that can deal directly with the challenge of a species that may remain undetected because of unsuitable sampling conditions. • Coordinating with the Department of Forestry on their forestry practices that may not be suitable for the management of flatwoods salamander populations. • Confidence in science.

Table 16. (continued)

QUESTION	SUMMARY OF ANSWERS
Is uncertainty in sampling effectiveness (i.e., detectability) a significant concern to you or your organization? Why?	• Yes. There must be some standardized method to determine at what point you can stop surveying and be confident that they are not present. • Yes. Need to know when a site has been effectively surveyed to say there is little likelihood of presence. • Yes. When faced with the difficulty of determining absence. • Yes. It makes it difficult to apply standardized sampling techniques and determine actual population size, thereby hindering applicability of sampling results across populations. • Yes. We don't know when to consider a population to no longer be extant and not be included any longer in management plans. • Yes. Because populations can be destroyed by development if imperfect sampling suggests that the species does not occur on-site. • Yes. Of course it would be best to know with 100% certainty; however, for a highly likely pond it is treated with caution. • Yes. Wetland habitats vary considerably. • No. Just need to know how the population is doing and what we need to ensure viability. • No. We will continue to manage our wetlands whether or not we detect salamanders. • No. It's reality.
What insight have you gained from this workshop that might affect how you manage or monitor flatwoods salamander populations?	• I will continue dipnetting and forget about minnow traps. • More knowledge on knowing how to better determine the most appropriate sampling effort and appropriate habitat type. • Number of visits necessary before a certain probability of absence is obtained. • Provided good information on sampling effectiveness, which methods are best, and how much effort is required to maximize detection probabilities. • It has helped me in determining ways that are best in monitoring for flatwoods salamander larvae detection. Might be beneficial to also include drift fences. • Sampling effort required at different detection levels. • Sampling is our biggest challenge to adaptive management. We need confidence to understand a species that has already disappeared from most of its historic sites. • We are starting to work toward methods that will give land managers standards to go by for surveying flatwoods salamanders. Also, it is apparent that protection on private land may be a major key in saving this species. • Specifying research objectives is critical – sometimes questions asked by managers can't be answered directly, but related issues may be easier to address. • Potential use of models and need for research. • More work needs to be done.

Table 16. (continued)

QUESTION	SUMMARY OF ANSWERS
To what other species that you or your organization works with might the ideas discussed today be applicable?	• Striped newts, eastern newts, marbled salamanders, tiger salamanders, spotted salamanders, dusky salamanders, four-toed salamanders, gopher frogs, ornate chorus frogs. • Many pond breeders. • Any larval amphibians. • Any amphibian. • Rare riverine turtles. • Rare bird species. • Panama City crayfish.

At the completion of both of our Florida sampling trips (Eglin AFB in 2006 and Apalachicola NF and St Marks NWR) we submitted trip reports to the organizations involved detailing our efforts and results.

The Knoxville, Tennessee newspaper The Knoxville News Sentinel ran a full page story on the project on June 20, 2005 (Appendix A.6).

7.4 SAMPLING RECOMMENDATIONS

Based on the results of our field work and modeling, we provide several recommendations for future survey work for flatwoods salamanders at Fort Stewart and across its range. Our recommendations address how, when, where, how often, and what to survey in order to maximize the detection of flatwoods salamander larvae. Appendix C provides a summary of recommendations that should be beneficial to those interested in detecting or monitoring flatwoods salamander larvae throughout the southeast.

How?

Our results suggest that dipnetting is the preferred method of sampling based primarily on catch per effort but also on material cost. It is possible that more effective traps could be developed that take advantage of specific flatwoods salamander behavior, but currently used traps are not as effective from a capture rate or cost perspective as dipnets. There are numerous combinations of various levels of effort and sampling techniques that could be used in a monitoring program, but because the primary expense is labor, the cost of any sampling plan is largely driven by how much investigator effort is invested.

When?

Capture of larvae is most successful when larvae are most abundant and large enough to be retained in dipnets and easily seen amongst the debris that is collected during each sweep. We suggest that this period is primarily during the second and third month after hatching. Hatching can best be estimated as being within a day or two of significant rises in pond elevation in

December through February. Hatching can occur as late as March and multiple hatching dates within a pond are common. We suggest that since pond filling that results in hatching is not easily determined, that one or two sentinel ponds that are most regularly productive be regularly monitored from December through March (or until larvae are found). Evidence of hatching in a representative and reliable pond (or ponds) should be used to dictate the best time to sample a large number of ponds. Hatching and metamorphosis typically occurs earlier in Florida populations than at Fort Stewart, but pond filling could conceivably reverse this occasionally. Based on the late residency observed in 2005, we suggest that sampling at Fort Stewart should not be stopped until the end of April if water is still present in the ponds.

Where?

When faced with more ponds to sample than available resources, the choice of which ponds to sample should be made in a systematic way. Our sampling simulation results suggest that if there is a known factor that correlates with salamander occupancy (e.g., proximity to a known pond, habitat similarity, presence or absence of predators/competitors, or food availability), then that factor or a suite of factors should be used to develop an adaptive sampling plan. For example, if a first round of sampling of randomly selected ponds results in detected occupancy in some ponds then subsequent sampling should be at those ponds that are most similar based on whatever factor is a significant correlate. Otherwise, if there are no significant correlates (including proximity) then continuing random selection of ponds for subsequent sampling is advised.

We used habitat modeling to predict probability of larval occurrence in ponds of unknown occupancy based on the characteristics of historically identified known breeding ponds. Although we can not be certain that habitat is a strong predictor of occupancy, we suggest that habitat along with proximity to known ponds should be considered when selecting ponds for sampling at Fort Stewart. Because the different habitat models we generated did not agree 100% as to which unknown ponds are most like the known ponds, we created an index that combines the results of three habitat models into a single score. The rank results from each model were standardized to a maximum score of 1 such that a pond that was ranked high by all three models would have a score of 3. The resulting ranking of ponds based on the combined index score provides a way to prioritize ponds at Fort Stewart for sampling based on similar habitat.

We tracked specific locations of captured larvae within the single detected pond at Fort Stewart to better understand where within the pond we might maximize effort, but came to no conclusions with regards to distance from edge of pond, water depth, or other pond characteristics. There are specific vegetation types that seasoned experts recognize as likely habitat for flatwoods salamander larvae and these are easily conveyed to inexperienced netters. This habitat includes primarily emergent grasses and other plants with slim vertical stalks or blades such as wiregrass (*Aristida* species), beakrush (*Rhynchospora* species), and hatpin (*Eriocaulon* species).

How often?

One goal of the capture probability and simulated sampling analyses described in Chapter 6 was to determine how to distribute sampling effort among ponds and how many times a pond should

be sampled following repeated non-detects. These are two very different questions. The simulated sampling exercise suggests that if there is no basis for adaptive sampling then random selection of ponds is adequate and is most effective if ponds are only sampled once or twice before moving on to other ponds. The amount of sampling necessary to achieve confidence in declaring that a pond is unoccupied requires an estimate of detection probability. If larvae are detected at other ponds at the same location then one might choose to use the average detection probability for unknown ponds. Alternatively, it would be more conservative to use the lowest detection probability observed at other ponds for the unknown ponds. Once a detection probability is chosen, the number of surveys necessary to achieve a specified level of confidence can be selected from Figure 37 or easily calculated. For example, the lowest P[detection] (see proportion detected in Table 15) from the Fort Stewart pond was 0.07 for a 5-min sampling period. This converts to a 60-min P[detection] of 0.56. From Figure 37, we find that at a P[detection] of 0.56 would require about 4 consecutive 1-hr surveys without finding a salamander larva to be 95% confident that a pond is unoccupied. From Figure 17 there are six known ponds that have been sampled at least once in 4 years since the 3-year drought that ended in 2002 without a detection. Another seven ponds have been sampled unsuccessfully 3 times during that time. Although the probability that a pond was occupied and not detected for any one of those years is 0.44 (i.e., 1 - 0.56), the odds that they were occupied every year and not detected every year are very small.

For Fort Stewart, conservation and monitoring goals for flatwoods salamanders are included in the Integrated Natural Resources Management Plan: Multi-species Endangered Species Management Plan (2001-2005). Monitoring requirements are stated as follows:

> "Annual monitoring of flatwoods salamander populations on Fort Stewart will be initiated in FY 2000 at 10 known breeding sites. Most other recently documented salamander breeding sites, as well as ponds at or near historic sites where the actual breeding pond was not identified will be sampled biennially, beginning in FY 2000. Other documented sites (ditches, wet firebreaks, etc.) will be monitored opportunistically. … In drought years, monitoring may not be possible at some or all sites.
>
> At sites monitored annually, dipnet or minnow trap sampling will be conducted 1-2 times a year during February - March to survey flatwoods salamander larvae. Biennial monitoring will involve dipnet surveys February-March to determine larval presence or absence. In order to document new flatwoods salamander breeding sites, additional ponds located within salamander Habitat Management Units will be sampled as time permits."

These guidelines require disproportionate sampling at already confirmed ponds. Annual sampling of known ponds is useful if the primary interest is to monitor existing populations, but it takes away from effort that could be spent looking for other occupied ponds. We suggest that if the primary goal is to identify additional breeding sites then some of the annual effort intended for known ponds should instead be used to sample unknown ponds more frequently than biennially. On the other hand, because of unsuccessful annual sampling of most of the known Fort Stewart ponds for the past several years, we are aware that the populations at Fort Stewart may be undergoing serious decline.

As for the February to March suggested sampling period, we suggest that April be included in the sampling period during most years, and that precipitation and pond filling be closely monitored. We found that rain events of 20 mm a day or 25 mm in a week appear to be enough to trigger hatching at least in the Alpha pond, and recommend that surveys be scheduled with recognition of rain events likely to initiate pond filling.

The high number of larvae found in several ponds in near proximity at St Marks NWR in 2007 suggests that meta-populations respond similarly and annual variation in larvae density is similar among ponds. Given this observation, an efficient sampling approach for Fort Stewart might be to distribute effort unevenly among years, expending as much as possible in years when conditions and sampling at dependable ponds suggests that it might be a banner year.

The Florida Fish and Wildlife Conservation Commission (FFWCC) has established flatwoods salamander management criteria for populations throughout the state. General guidelines include the following statements:

- The objective of the FFWCC is to confirm the presence of flatwoods salamanders at known sites at least once every five years

- If dipnet surveys of potential breeding sites are unsuccessful in >3 good sampling years, which may occur over a period of >10 years, additional sampling will not be needed because the wetlands presumably are not current breeding sites.

This plan basically includes a stopping rule of three. From Figure 36 we see that to be 90% confident that 3 non-detects in a row did not occur in an occupied pond would require densities resulting in a detection probability of >0.55. If one assumes each of these surveys lasts an hour, then a 60-min P[detection] = 0.55 corresponds to a 5-min p[detection] = $1-[1-0.55]^{1/12} = 0.064$. Of the 8 Florida ponds where we found larvae in 2006, all exceeded a 5-min p[detection] of 0.064 (Table 15), which suggests that a stopping rule of three is reasonable as long as the years included in the sampling are "good sampling years". In their management plan, the FFWCC defines a good sampling year as one in which rainfall has completely filled wetland basins and inundated ecotonal edges by 1 January, and larval salamanders are present at other wetlands in the region.

What?

While sampling to detect larval presence, there is a suite of information that can be collected at the same time that will provide useful data for understanding many of the questions surrounding flatwoods salamander detection. Survey time should be closely monitored with periodic stops (e.g., every 5 min) to record the number of larvae captured for each netter. If possible, noting the exact time of each capture is even better. These data can be used to estimate detection probability. Snout-vent and total lengths should be recorded so that estimates of pond residency (hatch date and time to metamorphosis) can be calculated. Pond elevation or pond depth should be measured every trip to a pond for a better understanding of pond hydroperiod. Although collecting enough data for a habitat analysis takes a lot of effort, some observations should be made at least annually at each pond visited. Any noticeable changes in species composition or

abundance should be noted for the pond and surrounding upland, especially if any exotic or invasive species appear or increase in abundance. A more detailed habitat analysis once every 4 to 5 years at select known ponds using the procedures used in this project would provide valuable data for evaluating long-term changes in pond plant communities that might significantly impact the ponds' ability to support salamander reproduction.

7.5 OTHER MANAGEMENT RECOMMENDATIONS

The apparent decline of flatwoods salamanders at Fort Stewart requires further research directed at enhancing recruitment in areas where populations still persist and restoring populations in areas where they have previously occurred. The importance of conservation efforts for flatwoods salamanders at Fort Stewart cannot be overemphasized. This species has not been documented at other known wetland sites in Georgia since 1999, and a recent genetic analysis of this species suggests that populations in Georgia may be evolutionary distinct units from populations in northeastern Florida. Loss of populations at Fort Stewart would be a serious setback to efforts to maintain the genetic diversity of populations across the range of this species.

Enhancement of existing populations could be accomplished through rearing larval salamanders in cattle tanks. Larval salamander populations are assumed to undergo significant reductions in number from the time eggs are laid to the point when larvae complete metamorphosis and emerge from wetlands. Rearing larval salamanders in cattle tanks would hedge against ponds drying too quickly. Similar efforts have provided significant enhancements to a remnant population of dusky gopher frogs (*Rana sevosa*). Many other *Ambystoma* salamander species have been successfully reared in cattle tanks. Rearing efforts at Fort Stewart could test methods with another *Ambystoma* species like marbled salamanders (*Ambystoma opacum*) or tiger salamanders (*Ambystoma tigrinum*) before being implemented with flatwoods salamanders. Experimental releases of juvenile salamanders reared in cattle tanks could also be used to re-establish populations in areas where they appear to be extinct. It might be possible for such populations to be given less restrictive regulatory conditions if the sources of these experimental populations were maintained. Because attempts at restoration are likely only to take place in a year when resident populations are high and because this is likely a rare and unpredictable event, we recommend that Fort Stewart managers begin considerations of restoration soon and have a plan pre-approved so that when the opportunity arises to relocate some individuals it can be done in a timely manner.

Restoration efforts should be informed by knowledge about genetic diversity of populations across Fort Stewart, factors associated with salamander movement and dispersal, and the status of other biotic threats to larval amphibians such as potential competitors, predators, and pathogens. Since flatwoods salamander populations appear to be greatly restricted in their distribution, genetic analyses of other salamander species such as eastern newts (*Notophthalmus viridescens*), striped newts (*Notophthalmus perstriatus*), dwarf salamanders (*Eurycea quadridigitata*), and tiger salamanders (*Ambystoma tigrinum*) would reveal variation in genetic structure across Fort Stewart. In the absence of other alternatives, populations at Fort Stewart could be reestablished from one area on the base where the species still persists, but it would be most desirable to reestablish populations from sources that were more genetically similar and

avoid homogenizing the genetic diversity of the species through reintroductions from a single source population. Genetic information would also provide a quantitative assessment of the populations currently identified in Fort Stewart's Integrated Natural Resources Management Plan; it is possible that there is more fine-scale genetic variation within those populations that could be worth maintaining.

7.6 THREATS TO FLATWOODS SALAMANDER CONSERVATION

Surveys during the past 15-20 years at Fort Stewart and elsewhere in Georgia and South Carolina suggest that many populations have declined throughout these states and perhaps disappeared. In a questionnaire distributed during our end of project workshop, flatwoods salamander biologists from throughout the southeast ranked the possible causes for these declines in the following order (most to least likely):

- Habitat degradation
- Population fragmentation
- Drought
- Water quality
- Disease

An initial goal of the habitat modeling task was to evaluate possible effects of base activities on salamander presence and abundance. Base activities, such as construction and maintenance of dirt roads, ditching to control water runoff, heavy equipment training, and range development, could conceivably affect salamander populations by four of these six factors. We included distance to nearest road as one of the landscape features in our habitat model and the inclusion of the landscape features improved the predicative ability of the model. Fortunately, since most of the flatwoods salamander ponds are within or near areas managed for another protected species (red-cockaded woodpeckers), the upland habitat around flatwoods salamander ponds is maintained by regular burning.

Several ponds or wetlands contain features that are the result of human activities, including skidder tracks from past logging operations and fire breaks constructed to control wildfires. Although unnatural, these features often provide habitat for salamander larvae especially when ponds dry early and the only water left is contained in these depressions.

7.7 APPLICATION TO OTHER SPECIES

The methods we developed for flatwoods salamanders can be applied to sampling designs for a variety of rare organisms on military installations and elsewhere. Biologist that attended our end-of-project workshop identified a variety of animals for which the approach presented here might

be helpful in surveying (see the last question in Table 16). In addition to rare native species, the challenge of identifying the location of non-native invasive species is also a problem that can benefit from an approach that is more effective and reduces uncertainty. And, the approach can be equally effective whether the area of search is a natural ecosystem or a freight container that might be harboring an unwanted pest. Regardless of the search target, the key parameter necessary to evaluate effectiveness is the probability of detection. We presented two ways to estimate probability of detection, either of which is likely effective for a variety of circumstances.

7.8 FUTURE RESEARCH NEEDS

There is much that is unknown about the basic biology of flatwoods salamanders, especially with regard to adult habitat use and breeding migrations. Movement and dispersal of salamanders could be examined through a few different methods. Tracking individuals with dye powder is one way to obtain information about movement over short distances (Roe and Grayson 2008). While radiotransmitters are problematic on small animals, continued advances in telemetry technology may make the prospect of radiotracking flatwoods salamanders more feasible in the future, and the use of harmonic radar remains one alternative to radiotelemetry that is expensive and has been successfully used to track insects.

Better information on where and when eggs are laid would add much to our understanding of the timing of hatching relative to pond filling and the types of conditions that promote hatching success and larval survival. Little is known about the preferred micro-habitat for egg deposition or how subtle changes in elevation factor into site selection for egg deposition. Understanding these factors is important in understanding the impacts of artificial land features around and through some ponds, such as fire breaks, logging tracks, and training bunkers.

Finally, the status of other potential biotic threats to flatwoods salamanders throughout its range such as competitors, predators, and pathogens should be explored. Direct experimentation with rare larval amphibians such as flatwoods salamanders and potential predators can be done in special venues. For example, larval behavior can be observed in the presence and absence of caged predators in small aquaria. Dietary studies on potential competitors could provide more information about the potential for competition between flatwoods salamanders and other amphibian or fish species that are found in wetlands where flatwoods salamanders have occurred. Finally, the status of potential pathogens remains largely unknown in many areas, but this could present a significant obstacle to conservation efforts. The presence of pathogens in both temporary and permanent waters should be assessed since some species recolonize temporary ponds from permanent water sources. Strict protocols should also be followed to ensure that research and monitoring activities do not increase the risk of spreading diseases among wetlands.

8. LITERATURE CITED

Anderson, J.D., and G.K. Williamson. 1976. Terrestrial mode of reproduction in *Ambystoma cingulatum*. Herpetologica 32(2):214-21.

Baber, M. J., D. L. Childers, K. J. Babbitt, and D. H. Anderson. 2002. Controls on fish distribution and abundance in temporary wetlands. Canadian Journal of Fisheries and Aquatic Sciences 59: 1441-1450.

Biek, R., W. C. Funk, B. A. Maxell, and L. S. Mills. 2002. What is missing in amphibian decline research: insights from ecological sensitivity analysis. Conservation Biology 16: 728-734.

Binckley, C. A., and W. J. Resetarits. 2002. Reproductive decisions under threat of predation: squirrel treefrog (*Hyla squirella*) responses to banded sunfish (*Enneacanthus obesus*). Oecologia 130: 157-161.

Bishop, D.C., and C.A. Haas. 2005. Burning trends and potential negative effects of suppressing wetland fires on Flatwoods Salamanders. Natural Areas Journal 25:290-294.

Bishop, D.C., J.G. Palis, K.M. Enge, D.J. Printiss and D.J. Stevenson. 2006. Capture rate, body size, and survey recommendations for larval *Ambystoma cingulatum* (Flatwoods Salamanders). Southeastern Naturalist 5(1):9-16.

Boone, M. D., R. D. Semlitsch, J. F. Fairchild, and B. B. Rothermel. 2004. Effects of an insecticide on amphibians in large-scale experimental ponds. Ecological Applications 14: 685-691.

Buech, R. R., and L. M. Egeland. 2002. A comparison of the efficacy of survey methods for amphibians breeding in small forest ponds. Herpetological Review 33(4):275-280.

Carle, F. L. and M. R. Strub. 1978. A new method for estimating population size from removal data. Biometrics 34:621-630.

Carlile, L.D. 1995. Fire effects on threatened and endangered species and habitats of Fort Stewart Military Reservation, Georgia. Pp. 227-231, In J.M. Greenlee (Ed.). Proceedings: Fire Effects on Rare and Endangered Species and Habitats Conference. International Association of Wildland Fire, Coeur d’ Alene, ID. 343 pp.

Gawin, L., K. Lutz, D. Mikesic, and D. Stevenson. 1995. Fort Stewart inventory final report. The Nature Conservancy, GA Field Office, Pembroke, GA.

Green, R. H., and R. C. Young. 1993. Sampling to detect rare species. Ecological Applications 3(2):351-356.

Gregoire, D. R., and M. S. Gunzburger. 2008. Effects of predatory fish on survival and behavior of larval gopher frogs (Rana capito) and southern leopard frogs (Rana sphenocephala). Journal of Herpetology 42: 97-103.

Grevstad, F.S. 2005. Simulating control strategies for a spatially structure weed invasion: *Spartina alterniflora* (Loisel) in Pacific Coast estuaries. Biological Invasions 7:665-677.

Gulve, P.S. 1994. Distribution and extinction patterns within a northern metapopulation of the pool frog, *Rana lessonae*. Ecology 75: 1357-1367.

Hargrove, W.W., and F.M. Hoffman. 2004. The potential of multivariate quantitative methods for delineation and visualization of ecoregions. Environmental Management 34(5):S39-S60.

Hargrove, W. W., F. M. Hoffman, and B. E. Law. 2003. New analysis reveals representativeness of the AmeriFlux Network. Eos 84: 529-535.

Jensen, J.B. 1999. Flatwoods Salamander (*Ambystoma cingulatum*). Pp. 92-93 In T.W. Johnson, J.C. Ozier, J.L Bohannon, J.B. Jenson, and C. Skelton (Eds.). Protected Animals Of Georgia. Nongame-Endangered Wildlife Program, Georgia Department of Natural Resources, Wildlife Resources Division, Nongame Wildlife-Natural Heritage Section. Forsyth, GA. 247 pp.

Kery, M. 2002. Inferring the absence of a species – a case study of snakes. J. Wildl. Manage. 66(2):330-338.

Mazerolle, M. J. 2005. Drainage ditches facilitate frog movements in a hostile landscape. Landscape Ecology 20: 579-590.

McGarigal, K., S. Cushman, and S. Stafford. 2000. Multivariate statistics for wildlife and ecology research. Springer-Verlag, New York.

Moran, P.A.P. 1951. A mathematical theory of animal trapping. Biometrika 38:307-311.

Mushet, D.M., N. H. Euliss, B. H. Hanson, and S. G. Zodrow. 1997. A funnel trap for sampling salamanders in wetlands. Herpetological Review 28(3):132-133.

Noble, G.K., and M.K. Brady. 1933. Observations on the life history of the marbled salamander, *Ambystoma opacum*. Zoologica 11:89-132.

Paice, M.E.R., W. Day, L.J. Rew, and C.L. Howard. 1998. A stochastic simulation model for evaluating the concept of patch spraying. Weed Research 38:373-388.

Palis, J.G. 1995. Larval growth, development, and metamorphosis of *Ambystoma cingulatum* on the Gulf Coastal Plain of Florida. Florida Scientist 58:352-358.

Palis, J.G. 1996. Element stewardship abstract for *Ambystoma cingulatum* - flatwoods salamander. Natural Areas Journal 16:49-54.

Palis, J.G. 1997. Distribution, habitat, and status of the Flatwoods Salamander (*Ambystoma cingulatum*) in Florida, USA. Herpetological Natural History 5:53-65.

Palis, J.G. 2002. Distribution of potential habitat of the federally threatened Flatwoods Salamander (*Ambystoma cingulatum*) on Fort Stewart, Georgia. Unpublished report to the Fort Stewart Fish and Wildlife Branch (contract DAKF10-01-P-0265). 5 pp.

Palis, J.G., M.J. Aresco, and S. Kilpatrick. 2006. Breeding biology of a Florida population of *Ambystoma cingulatum* (Flatwoods Salamander) during a drought. Southeastern Naturalist 5(1):1-8.

Pauly, G.B., O. Piskurek, and H.B. Shaffer. 2007. Phylogeographic concordance in the southeastern United States: the flatwoods salamander, *Ambystoma cingulatum*, as a test case. Molecular Ecology 16(2):415-429.

Pechmann, J. H. K., D. E. Scott, R. D. Semlitsch, J. P. Caldwell, L. J. Vitt, and J. W. Gibbons. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. Science 253: 892-895.

Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press. Washington and London. 587 pp.

Petranka, J.W., and J.G. Petranka. 1981. On the evolution of nest site selection in the marbled salamander, *Ambystoma opacum*. Copeia 1981:387-391.

Reed, J. M. 1996. Using statistical probability to increase confidence of inferring species extinction. Conservation Biology 10(4):1283-1285.

Roe, A. W., and K. L. Grayson. 2008. Terrestrial movements and habitat use of juvenile and emigrating adult eastern red-spotted newts, *Notophthalmus viridescens*. Journal of Herpetology 42:22-30.

Rothermel, B. B., and R. D. Semlitsch. 2002. An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. Conservation Biology 16: 1324-1332.

Ruetz, C. R., J. C. Trexler, F. Jordan, W. F. Loftus, and S. A. Perry. 2005. Population dynamics of wetland fishes: spatio-temporal patterns synchronized by hydrological disturbance? Journal of Animal Ecology 74: 322-332.

Schmidt, B. R., and J. Pellet. 2005. Relative importance of population processes and habitat characteristics in determining site occupancy of two anurans. Journal of Wildlife Management 69: 884-893.

Sekerak, C.M., G.W. Tanner, and J.G. Palis. 1996. Ecology of Flatwoods Salamander larvae in breeding ponds in Apalachicola National Forest. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 50:321-330.

Skelly, D. K. 1992. Field evidence for a cost of behavioral antipredator response in a larval amphibian. Ecology 73: 704-708.

Skelly, D. K. 1995. A behavioral trade-off and its consequences for the distribution of *Pseudacris* treefrog larvae. Ecology 76: 150-164.

Skelly, D. K., L. K. Freidenburg, and J. M. Kiesecker. 2002. Forest canopy and the performance of larval amphibians. Ecology 83: 983-992.

Smith, M. A., and D. M. Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography 28: 110-128.

Snodgrass, J. W., A. L. Bryan, Jr., R. F. Lide, and G. M. Smith. 1996. Factors affecting the occurrence and structure of fish assemblages in isolated wetlands of the upper coastal plain, U.S.A. Canadian Journal of Fisheries and Aquatic Sciences 53: 443-454.

Stevenson, D. J. 1999. The herpetofauna of Fort Stewart, Georgia: habitat occurrence, status of protected and rare species, and species diversity. Unpublished report to the Fort Stewart Fish and Wildlife Branch. 98 pp.

Sun, G., S. G. McNulty, J. P. Shepard, D. M. Amatya, H. Riekerk, N. B. Comerford, W. Skaggs, and L. Swift. 2001. Effects of timber management on the hydrology of wetland forests in the southern United States. Forest Ecology and Management 143: 227-236.

Thurgate, N. Y., and J. H. K. Pechmann. 2007. Canopy closure, competition, and the endangered dusky gopher frog. Journal of Wildlife Management 71: 1845-1852.

USFWS (U.S. Fish and Wildlife Service). 1999. Final Rule To List the Flatwoods Salamander as a Threatened Species. Federal Register 01-APR-99 64 FR 15691-15704.

U.S. Fish and Wildlife Service. 1999. Endangered and threatened wildlife and plants: final rule to list the flatwoods salamander as a threatened species. Federal Register 64: 15691.

Van Buskirk, J. 2005. Local and landscape influence on amphibian occurrence and abundance. Ecology 86: 1936-1947.

Wald, A. 1947. Sequential Analysis. New York: Wiley.

Wellborn, G. A., D. K. Skelly, and E. E. Werner. 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics 27: 337-363.

White, G. C., D. R. Anderson, K. E Burnham, and D. L. Otis. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory, Los Alamos, New Mexico.

White, D., K. Freemark, E. Preston, and R. Kiester. 1999. A hierarchical Framework for conserving biodiversity. Pages 127-153 in J.M. Klopatek and R. H. Gardner (eds.) Landscape Ecological Analysis: Issues and Applications. Springer-Verlag, Berlin.

Wilbur, H. M. 1997. Experimental ecology of food webs: complex systems in temporary ponds. Ecology 78: 2279-2302.

Williamson, G.K. and R.A. Moulis. 1979. Survey of reptiles and amphibians on Fort Stewart and Hunter Army Airfield. Report to U.S. Army, Fort Stewart, GA (contract DACA 21-77-c-0155).

Zippin, C. 1958. The removal method of population estimation. Journal of Wildlife Management 22:82-90.

APPENDIX A. SUPPORTING DATA

A.1 FORT STEWART FLATWOODS SALAMANDER MONITORING PLAN 2000-2005

A.2 FLATWOODS SALAMANDER CAPTURE INFORMATION

A.3 LIST OF SURVEYED POND

A.4 HABITAT METADATA

A.5 SAMPLING MODEL CODE AND INPUT

A.6 KNOXVILLE NEWS SENTINEL ARTICLE

A.1 FORT STEWART FLATWOODS SALAMANDER MONITORING PLAN 2000-2005

From the Integrated Natural Resources Management Plan: Multi-species Endangered Species Management Plan (2001-2005).

Per the plan our (Ft Stewart) "conservation goal is to maintain the 5 existing populations and 21 breeding sites currently known on Fort Stewart, and to manage other areas of suitable habitat in a manner to encourage the establishment of viable salamander populations"

Flatwoods salamander:
Annual monitoring of flatwoods salamander populations on Fort Stewart will be initiated in FY 2000 at 10 known breeding sites. Most other recently documented salamander breeding sites, (Gawin et al. 1995) as well as ponds at or near historic sites where the actual breeding pond was not identified (Williamson and Moulis 1979) will be sampled biennially, beginning in FY 2000. Other documented sites (ditches, wet firebreaks, etc.) will be monitored opportunistically. Currently, Georgia St. Univ. is monitoring 1 salamander breeding site with a drift fence. Monitoring protocols have been scheduled for all breeding sites (Table 7). In drought years, monitoring may not be possible at some or all sites.

At sites monitored annually, dipnet or minnow trap sampling will be conducted 1-2 times a year during February - March to survey flatwoods salamander larvae. Biennial monitoring will involve dipnet surveys February-March to determine larval presence or absence. In order to document new flatwoods salamander breeding sites, additional ponds located within salamander Habitat Management Units will be sampled as time permits.

Flatwoods Salamander Sites and Monitoring Protocols.

Flatwoods Salamander Sites on Fort Stewart

Site Code #	Description	Observation Dates				Monitoring Protocol
A4.1-01	crossing hwy.	1975(A)				opportunistically
A4.2-01	cypress pond	1995(L)	1996(A)	1997(A)		biennial
A6.4-01	roadside ditch	1994(L)				opportunistically
A6.4-02	gum pond	1994(L)				annual
A6.4-03	gum pond	1994(L)				biennial
A6.4-04	cypress pond	1993(A)	1994(L)	1997(A,L)		annual
A6.4-05	cypress depression	1994(L)	1995(L)	1996(A)		biennial
A7.2-01	borrow pit	1994(L)				opportunistically
A10.2-01	cypress pond	1994(L)	1997(A,L)			annual*
A10.2-02	cypress pond	1994(L)				annual
A10.2-03	borrow pit	1994(L)	1994(A)			opportunistically
A18.1-01	crossing hwy.	1975(A)				opportunistically
B4.10-01	cypress pond	1975(A)				biennial
B19.4-01	cypress pond / flooded road	1994(L)	1997(L)			annual
B20.2-01	cypress pond	1994(L)				annual
B20.3-01	roadside ditch	1994(L)				opportunistically
D12.1-01	crossing road	1979(A)				opportunistically
E1.3-01	crossing road	1977(A)				opportunistically
E3.2-01	crossing road	1976(A)				opportunistically
E10.1-01	cypress pond	1994(L)				biennial
E10.1-02	cypress pond	1994(L)				annual
E11.2-01	cypress pond	1994(L)	1997(L)			biennial
E11.2-02	cypress pond	1994(L)	1997(L)			annual
E11.5-01	cypress pond	1978(A)				biennial
F6.3-01	cypress pond	1992(A)				annual
F7.2-01	cypress pond	1994(L)				annual
F7.2-02	cypress pond	1994(L)				biennial
F7.4-01	cypress pond	1995(L)				biennial
F9.5-01	gum pond	1978(L)				biennial
F9.5-02	cypress pond	1978(A)				biennial

A=flatwoods salamander adult(s) observed
L=flatwoods salamander larva(e) observed
N/A=suitable flatwoods habitat not present at this site
* Site currently monitored by GSU

Larvae captured will be measured (snout-vent length and total length in mm) and weighed (to the nearest 0.1 g) after being placed in a plastic bag with a small amount of water. For each larva collected, distance from shore, depth, and dominant plant species at the collection site will be recorded. The following data will be recorded on each salamander survey: air temperature, water temperature, pH, maximum depth, number of dipnet sweeps or minnow trap-nights, and other aquatic fauna observed.

A.2 FLATWOODS SALAMANDER CAPTURE INFORMATION

Table A9.2. Location, date, method, and size information for larval flatwoods salamanders captured during the course of the study at Ft Stewart, Eglin, St Marks, and Apalachicola

Pond	Date	Method	SVL (mm)	TL (mm)
Fort Stewart				
A10.2-02	4/21/2005	plastic trap	35	62
A10.2-02	4/21/2005	dipnet	37	70
A10.2-02	4/21/2005	dipnet	35	69
A10.2-02	4/21/2005	dipnet	37	68
A10.2-02	4/21/2005	dipnet	31	63
A10.2-02	4/21/2005	dipnet	35	64
A10.2-02	4/21/2005	dipnet	28	52
A10.2-02	4/21/2005	dipnet	29	59
A10.2-02	4/21/2005	dipnet	29	62
A10.2-02	4/21/2005	dipnet	25	50
A10.2-02	4/21/2005	dipnet	36	66
A10.2-02	4/21/2005	dipnet	33	66
A10.2-02	4/21/2005	dipnet	32	55
A10.2-02	4/21/2005	dipnet	31	56
A10.2-02	4/21/2005	dipnet	27	54
A10.2-02	5/2/2005	dipnet	29	48
A10.2-02	5/2/2005	dipnet	34	61
A10.2-02	5/2/2005	dipnet	32	69
A10.2-02	5/2/2005	dipnet	35	61
A10.2-02	5/2/2005	dipnet	36	62
A10.2-02	5/2/2005	dipnet	36	69
A10.2-02	5/2/2005	dipnet	35	65
A10.2-02	5/2/2005	dipnet	34	60
A10.2-02	5/2/2005	dipnet	35	65
A10.2-02	5/2/2005	dipnet	36	62
A10.2-02	5/2/2005	dipnet	35	64
A10.2-02	5/3/2005	dipnet	34	67
A10.2-02	5/3/2005	dipnet	36	66
A10.2-02	5/4/2005	metal trap	36	66
A10.2-02	5/4/2005	metal trap	32	56
A10.2-02	5/11/2005	dipnet	28	42
A10.2-02	5/11/2005	dipnet	30	46
A10.2-02	5/11/2005	dipnet	34	59
A10.2-02	5/11/2005	dipnet	34	63
A10.2-02	5/11/2005	dipnet	34	61
A10.2-02	5/17/2005	dipnet	32	56
A10.2-02	5/17/2005	dipnet	35	63
A10.2-02	5/23/2005	dipnet	32	58
A10.2-02	01/18/06	dipnet	15	26
A10.2-02	01/18/06	dipnet	16	28
A10.2-02	01/27/06	dipnet	16	22
A10.2-02	02/07/06	dipnet	15	26

Pond	Date	Method	SVL (mm)	TL (mm)
A10.2-02	02/08/06	dipnet	17	23
A10.2-02	02/08/06	dipnet	20	40
A10.2-02	02/08/06	dipnet	18	37
A10.2-02	02/08/06	dipnet	19	35
A10.2-02	02/08/06	dipnet	18	34
A10.2-02	02/08/06	dipnet	18	30
A10.2-02	02/08/06	dipnet	16	30
A10.2-02	02/08/06	dipnet	15	28
A10.2-02	02/08/06	dipnet	15	35
A10.2-02	02/08/06	dipnet	15	35
A10.2-02	03/08/06	dipnet	30	48
A10.2-02	03/08/06	dipnet	28	60
A10.2-02	03/08/06	dipnet	30	52
A10.2-02	03/08/06	dipnet	26	38
A10.2-02	03/08/06	dipnet	30	50
A10.2-02	03/08/06	dipnet	29	53
A10.2-02	03/08/06	dipnet	33	54
A10.2-02	04/04/06	dipnet	28	53
A10.2-02	04/04/06	dipnet	37	65
A10.2-02	04/04/06	dipnet	29	43
A10.2-02	04/04/06	dipnet	32	58
A10.2-02	04/04/06	dipnet	31	43
A10.2-02	04/04/06	dipnet	33	55
A10.2-02	02/27/08	dipnet	27	51.4
A10.2-02	02/27/08	dipnet	25.4	37.2
A10.2-02	02/27/08	dipnet	20.25	38.1
A10.2-02	02/27/08	dipnet	25.5	55.1
A10.2-02	02/27/08	dipnet	18.2	39.5
A10.2-02	02/27/08	dipnet	20.6	40
A10.2-02	02/27/08	dipnet	15.2	35.9
A10.2-02	02/27/08	dipnet	18.2	38
A10.2-02	02/27/08	dipnet	17.5	40
		Eglin AFB		
16	3/14/2006	dipnet	34	53
16	3/14/2006	dipnet	36	55
16	3/14/2006	dipnet	39	77
16	3/14/2006	dipnet	33	60
16	3/14/2006	dipnet	34	62
16	3/14/2006	dipnet	33	47
16	3/14/2006	dipnet	37	59
16	3/14/2006	dipnet	39	68
16	3/14/2006	dipnet	35	64
16	3/14/2006	dipnet	33	57
16	3/14/2006	dipnet	38	63
		St. Marks NWR		
SMNWR 0110	2/22/2007	dipnet	38	62
SMNWR 0110	2/22/2007	dipnet	32	66
SMNWR 0110	2/22/2007	dipnet	36	64

Pond	Date	Method	SVL (mm)	TL (mm)
SMNWR 0110	2/22/2007	dipnet	32	62
SMNWR 0110	2/22/2007	dipnet	35	70
SMNWR 0110	2/22/2007	dipnet	28	48
SMNWR 0110	2/22/2007	dipnet	29	69
SMNWR 0110	2/22/2007	dipnet	31	64
SMNWR 0110	2/22/2007	dipnet	27	55
SMNWR 0110	2/22/2007	dipnet	37	70
SMNWR 0110	2/22/2007	dipnet	42	80
SMNWR 0110	2/22/2007	dipnet	35	71
SMNWR 0110	2/22/2007	dipnet	36	74
SMNWR 0110	2/22/2007	dipnet	38	53
SMNWR 0110	2/22/2007	dipnet	26	43
SMNWR 0110	2/22/2007	dipnet	38	57
SMNWR 0110	2/22/2007	dipnet	39	75
SMNWR 0110	2/22/2007	dipnet	31	70
SMNWR 0110	2/22/2007	dipnet	23	38
SMNWR 0110	2/22/2007	dipnet	34	65
SMNWR 0110	2/22/2007	dipnet	32	67
SMNWR 0110	2/22/2007	dipnet	34	64
SMNWR 0110	2/22/2007	dipnet	25	52
SMNWR 0110	2/22/2007	dipnet	34	70
SMNWR 0110	2/22/2007	dipnet	30	61
SMNWR 0110	2/22/2007	dipnet	33	62
SMNWR 0110	2/22/2007	dipnet	31	55
SMNWR 0110	2/22/2007	dipnet	31	64
SMNWR 0110	2/22/2007	dipnet	30	62
SMNWR 0110	2/22/2007	dipnet	32	72
SMNWR 0110	2/22/2007	dipnet	31	65
SMNWR 0110	2/22/2007	dipnet	27	48
SMNWR 0110	2/22/2007	dipnet	30	64
SMNWR 0110	2/22/2007	dipnet	32	68
SMNWR 0110	2/22/2007	dipnet	31	68
SMNWR 0110	2/22/2007	dipnet	27	63
SMNWR 0110	2/22/2007	dipnet	35	69
SMNWR 0110	2/22/2007	dipnet	32	65
SMNWR 0110	2/22/2007	dipnet	33	66
SMNWR 0110	2/22/2007	dipnet	32	65
SMNWR 0110	2/22/2007	dipnet	29	60
SMNWR 0110	2/22/2007	dipnet	32	67
SMNWR 0110	2/22/2007	dipnet	24	47
SMNWR 0110	2/22/2007	dipnet	28	56
SMNWR 0110	2/22/2007	dipnet	27	34
SMNWR 0108	2/22/2007	dipnet	31	65
SMNWR 0108	2/22/2007	dipnet	32	64
SMNWR 0108	2/22/2007	dipnet	33	70
SMNWR 0108	2/22/2007	dipnet	34	72
SMNWR 0108	2/22/2007	dipnet	35	80
SMNWR 0108	2/22/2007	dipnet	36	70
SMNWR 0108	2/22/2007	dipnet	36	82

Pond	Date	Method	SVL (mm)	TL (mm)
SMNWR 0108	2/22/2007	dipnet	36	68
SMNWR 0108	2/22/2007	dipnet	37	70
SMNWR 0108	2/22/2007	dipnet	37	84
SMNWR 0108	2/22/2007	dipnet	37	77
SMNWR 0108	2/22/2007	dipnet	38	80
SMNWR 0108	2/22/2007	dipnet	39	74
SMNWR 0108	2/22/2007	dipnet	39	84
SMNWR 0108	2/22/2007	dipnet	39	78
SMNWR 0108	2/22/2007	dipnet	40	80
SMNWR 0108	2/22/2007	dipnet	40	79
SMNWR 0108	2/22/2007	dipnet	40	82
SMNWR 0108	2/22/2007	dipnet	40	76
SMNWR 0108	2/22/2007	dipnet	41	84
SMNWR 0108	2/22/2007	dipnet	41	78
SMNWR 0108	2/22/2007	dipnet	42	85
SMNWR 0108	2/22/2007	dipnet	42	87
SMNWR 0108	2/22/2007	dipnet	43	84
SMNWR 0108	2/22/2007	dipnet	43	86
SMNWR 0108	2/22/2007	dipnet	43	85
SMNWR 0108	2/22/2007	dipnet	44	84
SMNWR 0108	2/22/2007	dipnet	44	88
SMNWR 0108	2/22/2007	dipnet	44	92
WAKDC 0009	2/20/2007	dipnet	36	74
WAKDC 0009	2/20/2007	dipnet	32	63
WAKDC 0009	2/20/2007	dipnet	32	64
WAKDC 0009	2/20/2007	dipnet	33	61
WAKDC 0009	2/20/2007	dipnet	29	58
WAKDC 0009	2/20/2007	dipnet	26	54
WAKDC 0009	2/20/2007	dipnet	29	59
WAKDC 0009	2/20/2007	dipnet	32	70
WAKDC 0009	2/20/2007	dipnet	33	62
WAKDC 0009	2/20/2007	dipnet	33	66
WAKDC 0009	2/20/2007	dipnet	31	56
WAKDC 0009	2/20/2007	dipnet	27	64
WAKDC 0009	2/20/2007	dipnet	36	72
WAKDC 0009	2/20/2007	dipnet	32	63
WAKDC 0009	2/20/2007	dipnet	30	64
WAKDC 0009	2/20/2007	dipnet	31	62
WAKDC 0009	2/20/2007	dipnet	35	70
WAKDC 0009	2/20/2007	dipnet	27	45
WAKDC 0009	2/20/2007	dipnet	44	88
WAKDC 0009	2/20/2007	dipnet	35	70
WAKDC 0009	2/20/2007	dipnet	34	65
WAKDC 0009	2/20/2007	dipnet	35	68
WAKDC 0009	2/20/2007	dipnet	31	62
WAKDC 0009	2/20/2007	dipnet	34	68
WAKDC 0009	2/20/2007	dipnet	35	67
WAKDC 0009	2/20/2007	dipnet	19	43
WAKDC 0009	2/20/2007	dipnet	26	57

Pond	Date	Method	SVL (mm)	TL (mm)
SMNWR 0110	2/24/2007	metal trap	29	61
SMNWR 0110	2/24/2007	metal trap	37	68
SMNWR 0110	2/24/2007	metal trap	31	65
SMNWR 0110	2/24/2007	metal trap	37	83
SMNWR 0110	2/24/2007	metal trap	34	76
SMNWR 0110	2/25/2007	metal trap	27	59
SMNWR 0110	2/25/2007	plastic trap	37	74
SMNWR 0110	2/25/2007	plastic trap	32	70
SMNWR 0110	2/25/2007	metal trap	26	53
SMNWR 0110	2/25/2007	plastic trap	45	89
SMNWR 0110	2/25/2007	plastic trap	44	92
SMNWR 0110	2/25/2007	metal trap	31	60
SMNWR 0110	2/25/2007	metal trap	43	85
SMNWR 0110	2/25/2007	metal trap	36	72
WAKDC 0009	2/24/2007	plastic trap	42	85
WAKDC 0009	2/24/2007	metal trap	33	66
WAKDC 0009	2/25/2007	plastic trap	33	68
WAKDC 0009	2/25/2007	metal trap	42	86
SMNWR 0111	2/24/2007	plastic trap	40	81
SMNWR 0111	2/24/2007	metal trap	43	82
SMNWR 0111	2/24/2007	plastic trap	35	75
SMNWR 0111	2/24/2007	metal trap	32	61
SMNWR 0111	2/24/2007	plastic trap	43	92
SMNWR 0111	2/24/2007	plastic trap	37	70
SMNWR 0111	2/24/2007	plastic trap	35	72
SMNWR 0111	2/24/2007	plastic trap	41	81
SMNWR 0111	2/25/2007	plastic trap	32	70
SMNWR 0111	2/25/2007	plastic trap	45	84
SMNWR 0111	2/25/2007	plastic trap	43	82
SMNWR 0111	2/25/2007	metal trap	32	57
SMNWR 0111	2/25/2007	plastic trap	40	60
	Apalachicola NF			
AP 28.002	2/21/2007	dipnet	26	56
AP 28.002	2/21/2007	dipnet	26	46
AP 28.002	2/21/2007	dipnet	24	48
AP 28.002	2/21/2007	dipnet	30	56
AP 28.002	2/21/2007	dipnet	18	33
AP 28.002	2/21/2007	dipnet	25	54
AP 28.002	2/21/2007	dipnet	20	36
AP 28.002	2/21/2007	dipnet	20	39
AP 28.002	2/21/2007	dipnet	26	52
AP 28.002	2/21/2007	dipnet	26	49
AP 28.002	2/21/2007	dipnet	22	43
AP 28.002	2/21/2007	dipnet	26	50
AP 28.002	2/21/2007	dipnet	24	44
AP 28.002	2/21/2007	dipnet	23	46
AP 28.002	2/21/2007	dipnet	25	48
AP 28.002	2/21/2007	dipnet	26	42

Pond	Date	Method	SVL (mm)	TL (mm)
AP 28.002	2/21/2007	dipnet	28	54
AP 28.002	2/21/2007	dipnet	15	35
AP 28.002	2/21/2007	dipnet	26	54
AP 28.002	2/21/2007	dipnet	22	43
AP 28.002	2/21/2007	dipnet	26	50
AP 28.002	2/21/2007	dipnet	24	56
AP 28.002	2/21/2007	dipnet	26	36
AP 28.002	2/21/2007	dipnet	26	51
AP 28.002	2/21/2007	dipnet	25	48
AP 28.002	2/21/2007	dipnet	26	52
AP 28.002	2/21/2007	dipnet	23	43
AP 28.002	2/21/2007	dipnet	24	48
AP 28.002	2/21/2007	dipnet	20	40
AP77.011	2/21/2007	dipnet	33	65
AP77.011	2/21/2007	dipnet	31	50
AP77.011	2/21/2007	dipnet	29	60
AP77.011	2/21/2007	dipnet	29	55
AP77.011	2/21/2007	dipnet	32	61
AP77.011	2/21/2007	dipnet	33	61
AP77.011	2/21/2007	dipnet	26	54
AP77.011	2/21/2007	dipnet	27	49
AP77.011	2/21/2007	dipnet	32	61
AP77.011	2/21/2007	dipnet	32	58
AP77.011	2/21/2007	dipnet	29	57
AP77.011	2/21/2007	dipnet	31	60
AP77.011	2/21/2007	dipnet	30	68
AP77.011	2/21/2007	dipnet	33	63
AP77.011	2/21/2007	dipnet	29	57
AP77.011	2/21/2007	dipnet	32	60
AP77.011	2/21/2007	dipnet	30	65
AP77.011	2/21/2007	dipnet	33	68

A.3 LIST OF SURVEYED POND

			2005				2006				2007		
Pond ID	Known		Net	Trap	Habitat		Net	Trap	Habitat		Net	Trap	Habitat
FT STEWART													
A4.2-09	X		X	X	X		X	X	X				X
A5.1-01					X				X				X
A5.1-02									X				
A5.1-03					X				X				
A5.3-04					X				X				X
A6.1-03					X				X				X
A6.1-04					X				X				X
A6.1-05					X				X				X
A6.1-06					X				X				X
A6.2-04					X				X				X
A6.4-01							X		X				
A6.4-02	X		X				X		X				
A6.4-03	X				X		X		X				X
A6.4-05	X		X	X	X		X		X				
A6.4-06	X		X	X	X		X						X
A6.4-08	X				X		X						
A6.4-09	X						X						
A7.1-01					X				X				X
A7.1-02					X				X				X
A7.1-04					X				X				X
A7.1-05							X		X				
A7.2-01					X		X						X
A7.2-02	X						X						
A7.2-05									X				
A8.0-02					X				X				X
A8.0-03					X				X				X
A10.2-01	X		X	X	X		X	X	X				X
A10.2-02	X		X	X	X		X	X	X				X
A10.2-07	X						X		X				
D12.1-01							X		X				
D12.1-02							X		X				
D12.1-03							X		X				
D12.1-04							X		X				
D12.3-01								X	X				
D12.3-02									X				
D12.3-08							X		X				
D13.1-01									X				
D13.1-02									X				
D14.1-02							X						
D14.2-01							X		X				
D14.2-02							X		X				
D14.2-03							X						
D14.2-06									X				
D14.2-07							X	X	X				
D14.2-08									X				
D14.2-11									X				
D15.1-01							X						
E10.2-02									X				

			2005				2006				2007		
Pond ID	Known		Net	Trap	Habitat		Net	Trap	Habitat		Net	Trap	Habitat
E10.2-03									X				
E10.3-01							X		X				
E10.3-04	X		X				X		X				
E10.3-05	X		X	X			X	X					
E10.3-06							X		X				
E10.3-08									X				
E10.3-10							X		X				
E10.3-11									X				
E11.2-01							X						
E11.2-02	X		X	X			X		X				
E11.2-04	X		X	X			X						
E11.2-05							X		X				
E11.2-07							X		X				
E11.3-01									X				
E17.1-01									X				
F6.1-01							X		X				
F6.1-02							X						
F6.2-01									X				
F6.2-02									X				
F6.2-03									X				
F6.2-05									X				
F6.2-06							X		X				
F6.2-08									X				
F6.3-02							X		X				
F6.3-03							X		X				
F6.3-04	X						X	X	X				X
F6.3-05							X		X				
F6.3-06							X		X				
F6.3-07							X		X				
F6.3-08									X				
F6.3-09							X						
F6.4-04							X		X				
F6.4-09							X		X				
F6.4-10							X		X				
F6.5-01							X						
F6.5-02							X		X				
F6.5-03							X		X				
F6.5-04							X		X				
F6.5-05									X				
F6.5-14									X				
F6.7-12									X				
F7.2-01								X	X				
F7.2-02	X						X	X	X				X
F7.2-04	X						X						
F7.2-05							X		X				
F7.2-06							X						
EGLIN													
14							X						
15							X						
16							X						

			2005				2006				2007		
Pond ID	Known		Net	Trap	Habitat		Net	Trap	Habitat		Net	Trap	Habitat
18							X						
19							X						
30							X						
ST MARKS													
SMNWR0106													X
SMNWR0107													X
SMNWR0108											X		X
SMNWR0109											X		X
SMNWR0110											X	X	X
SMNWR0111											X	X	X
SMNWR0112											X		X
SMNWR0114											X		
SMNWR0115											X		
SMNWR0118													X
SMNWR0121													X
SMNWR0124													X
SMNWR0137													X
SMNWR0139													X
SMNWR0142													X
WAKDC0007													X
WAKDC0009											X	X	X
WAKDC0010													X
WAKDC0014													X
WAKDC0042													X
WAKKE0001													X
WAKKE0017													X
WAKSH0001													X
WAKSH0004													X
SMNWR0106													X
SMNWR0107													X
SMNWR0108											X		X
SMNWR0109											X		X
SMNWR0110											X	X	X
SMNWR0111											X	X	X
SMNWR0112											X		X
SMNWR0114											X		
SMNWR0115											X		
SMNWR0118													X
SMNWR0121													X
SMNWR0124													X
SMNWR0137													X
SMNWR0139													X
SMNWR0142													X
WAKDC0007													X
APALACHICOLA													
27.003													X
28.002											X		X
72.04													X

			2005				2006				2007		
Pond ID	Known		Net	Trap	Habitat		Net	Trap	Habitat		Net	Trap	Habitat
76.001													X
77.003													X
77.004													X
77.009													X
77.01													X
77.011											X		X
77.012													X
77.023													X
77.026											X		X
77.029											X		
77.037													X
77.05													X
77.051													X
94.001													X
100.004													X
100.007													X
100.008													X
100.009													X
100.01													X
100.111											X		X
100.112													X
101.001													X
101.004													X
101.005													X
101.006													X

A.4 HABITAT METADATA

Metadata for data file 'allgroups.csv' used in habitat analyses.

pond: unique identifier for survey site
site: study area (stewart – Ft. Stewart, st. marks – St. Marks National Wildlife Refuge, apalachicola – Apalachicola National Forest)
presence: documented presence of *Ambystoma cingulatum* at survey site (1 – present, 0 – not documented)
absence: absence of *Ambystoma cingulatum* at survey site based on habitat assessment of sites at Ft. Stewart by Palis and Stevenson (1 – absent, 0 – not absent)
rank: qualitative ranking of habitat at Ft. Stewart by Palis and Stevenson

The following fields were cover estimates derived from field surveys of wetland plant communities:
andropogon: *Andropogon virginicus* (broomsedge); National Wetlands Inventory wetland indicator status (NWI indicator status) - facultative
carex: *Carex atlantica* (prickly bog sedge); NWI indicator status - facultative wetland; structure - graminaceous
dichanthel: *Dichanthelium* spp. (rosette grass); NWI indicator status – facultative; structure - graminaceous
gallberry: *Ilex coriacea* (gallberry); NWI indicator status - facultative wetland; structure - shrub
gratiola: *Gratiola* spp. (hedgehyssop): NWI indicator status - status variable; structure – no group
hatpin: *Eriocaulon* spp. (pipewort): NWI indicator status – obligate; structure - emergent
holly: *Ilex opaca* (American holly): NWI indicator status – facultative; structure - shrub
hypericum:*Hypericum* spp. (St. Johns-wort): NWI indicator status - status variable; structure - shrub
blueflag: *Iris tridentata* (savannah iris): NWI indicator status – obligate; structure – no group
ludwig: *Ludwigia* spp. (primrose-willow): NWI indicator status – obligate; structure – aq2
lyonia: *Lyonia lucida* (fetterbush): NWI indicator status - facultative wetland; structure - shrub
mermaidweed: *Proserpinaca palustris* (mermaidweed): NWI indicator status – obligate; structure – aq2
myrica: *Myrica cerifera* (waxmyrtle): NWI indicator status – facultative; structure - shrub
panicum: *Panicum virgatum* (switchgrass): NWI indicator status – facultative; structure - graminaceous
pickerelweed: *Pontederia cordata* (pickerelweed): NWI indicator status – obligate; structure – aq1
pitcherplant: *Sarracenia* spp. (pitcher plant): NWI indicator status – obligate; structure - emergent
poisonivy: *Toxicodendron radicans* (poison ivy): NWI indicator status – facultative; structure - vine
redbay: *Persea borbonia* (redbay): NWI indicator status - facultative wetland; structure - shrub
rhyncospera: *Rhynchospora* spp. (beakrush): NWI indicator status - status variable; structure - graminaceous

rubus: *Rubus* spp. (blackberry): NWI indicator status - status variable; structure - shrub
sagittaria: *Sagittaria* spp. (arrowhead): NWI indicator status – obligate; structure – aq1
sensfern: *Onoclea sensibilis* (sensitive fern): NWI indicator status - facultative wetland; structure - fern
smilax: *Smilax* spp. (greenbriar): NWI indicator status – facultative; structure - vine
xyris: *Xyris* spp. (yellow-eyed grass): NWI indicator status – obligate; structure - emergent
sphagnum: *Sphagnum* spp. (sphagnum): NWI indicator status - status variable; structure - moss
wiregrass: *Aristida* spp. (wiregrass): NWI indicator status - status variable; structure - graminaceous
sporobolis: *Sporobolis* spp. (dropseed): NWI indicator status - status variable; structure - graminaceous
pennywort: *Hydrocotyle* spp. (pennywort): NWI indicator status - status variable; structure – no group
titi: *Cyrilla racemiflora* (titi): NWI indicator status - facultative wetland; structure - shrub
lycopodium: *Lycopodium* spp. (clubmoss): NWI indicator status - status variable; structure - moss
bacopa: *Bacopa* spp. (waterhyssop): NWI indicator status - status variable; structure – aq2
butterwort: *Pinguicula* spp. (butterwort): NWI indicator status - status variable; structure – no group
sawgrass: *Cladium* spp. (sawgrass): NWI indicator status – obligate; structure - graminaceous
royalfern: *Osmunda regalis* (royal fern): NWI indicator status – obligate; structure - fern
cypress: *Taxodium distichum* (cypress): NWI indicator status – obligate; structure - tree
gum: *Nyssa sylvatica* (black gum): NWI indicator status – facultative; structure - tree
maple: *Acer rubrum* (red maple): NWI indicator status – obligate; structure - tree
mlh: *Ilex myrtifolia* (myrtle leaf holly): NWI indicator status - facultative wetland; structure - tree
slash: *Pinus elliotii* (slash pine): NWI indicator status - facultative wetland; structure - tree
wateroak: *Quercus nigra* (water oak): NWI indicator status – facultative; structure - tree
sweetgum: *Liquidambar styraciflua* (sweetgum): NWI indicator status – facultative; structure - tree

The following fields were derived by grouping plant cover estimates:
FAC: sum of cover estimates of NWI indicator status facultative
FACW: sum of cover estimates of NWI indicator status facultative wetland
OBL: sum of cover estimates of NWI indicator status obligate
Gram: sum of cover estimates of graminaceous species
Em: sum of cover estimates of emergent species
Aq1: sum of cover estimates of aquatic species group 1
Aq2: sum of cover estimates of aquatic species group 2
Fern: sum of cover estimates of ferns
Shrub: sum of cover estimates of shrub cover
Vine: sum of cover estimates of vines
Moss: sum of cover estimates of moss

The following fields were derived from GIS data described in the text:

Nearroad: distance to nearest road (m)
Totalroad: length of roads within 2km of pond
Nearstream: distance to nearest stream (m)
Totalstream: length of streams within 2 km of pond
Nearwetland: distance to nearest wetland (m)
Totalwetland: area of wetlands (ha) within 2 km of pond

A.5 SAMPLING MODEL CODE AND INPUT

```
#SPATIAL VAR V7b.r   (written by MSBevelhimer Feb 2008)

#INPUTS               #Input defaults produce BASELINE conditions
rows = 90             #rows in landscape grid including buffer
columns = 120         #columns in landscape grid including buffer
buffer = 8            #number of cells in no-pond buffer around pondscape
propponds = .04       #proportion of cells occupied with a pond
propocc = .1          #proportion of ponds occupied with salamanders
clumps = 3            #approximate number of clumps of ponds desired
stoprulemin=1         #no. of non-detects before giving up on a pond  (1 to 5)
stoprulemax=5         #no. of non-detects before giving up on a pond (set equal if only one test
desired)
pondssamp=50          #number of ponds visited per sampling trip
trips=10              #sampling trips or passes
reps=50
set.seed(4)           #use this line to set rndm # seed to repeat initial pondscape.

totalcells = rows*columns    #landscape grid dimensions

ttlpondssmpld=array(0,reps)
aaa=array(0,reps)
bbb=array(0,reps)
ccc=array(0,reps)
ddd=array(0,reps)
eee=array(0,reps)

SUMttlpondssmpld=matrix(0,4,5)     #array of detctprob X stoprule results
AVGaaa=matrix(0,4,5)          #array of detctprob X stoprule results
AVGbbb=matrix(0,4,5)          #array of detctprob X stoprule results
AVGccc=matrix(0,4,5)          #array of detctprob X stoprule results
AVGddd=matrix(0,4,5)          #array of detctprob X stoprule results
AVGeee=matrix(0,4,5)          #array of detctprob X stoprule results
STDaaa=matrix(0,4,5)          #array of detctprob X stoprule results
STDbbb=matrix(0,4,5)          #array of detctprob X stoprule results
STDccc=matrix(0,4,5)          #array of detctprob X stoprule results
STDddd=matrix(0,4,5)          #array of detctprob X stoprule results
STDeee=matrix(0,4,5)          #array of detctprob X stoprule results

###Create and populate landscape with ponds (1 or no ponds per cell) (1s & 0s)
#Assign each pond an ID number, 1 to n
pondscape = matrix(0, columns, rows)   #cells w/ 0 or 1 depending on pond presence
pondid = matrix(0, columns, rows)      #assigns pond number to cells with ponds
count=0
for (i in 1:columns){
```

```
  if (i>buffer & i<=columns-buffer){
    for (j in 1:rows){
      if (j>buffer & j<=rows-buffer){
        pondscape[i,j]= round(runif(1)-.5+propponds)
        if (pondscape[i,j]==1){
        count=count+1
        pondid[i,j] = count
} } } } }

###Populate occupied ponds with salamanders (1s & 0s)
#First pass to establish clump centers
pondsocc = matrix(0, columns, rows)
keyponds=sample(sum(pondscape),clumps)
for (h in 1:clumps) {
  pondsocc[which(pondid==keyponds[h], arr.ind=TRUE)]=1
}
image(pondscape+pondsocc)

#Second pass to populate neighboring ponds based on proximity to occupied (1s & 0s)
while (sum(pondsocc)<(propocc*sum(pondscape))) {
  occupancy=sum(pondsocc)/sum(pondscape)
  if (occupancy<propocc){
  proximity = matrix(0, columns, rows)
  for (i in 1:columns){
    if (i>buffer & i<=columns-buffer){
      for (j in 1:rows){
        if (j>buffer & j<=rows-buffer){
          if (sum(pondsocc[(i-7):(i+7),(j-7):(j+7)])>0){
            proximity[i,j] = 1
  } } } } }

  for (i in 1:totalcells) {
    if (pondsocc[i] <1){
      pondsocc[i]= round(proximity[i]*runif(1)+propocc-.5)*pondscape[i]
  } }

  image(pondscape+pondsocc)
} }
print(sum(pondsocc))
print(max(pondid))

obn=pondsocc*pondid
occsbynumber=sort(obn[obn>0])
occsvector=rep(c(0),sum(pondscape))
occsvector[occsbynumber]<-1
```

```
trip1detects=matrix(0, 4, 5); trip2detects=matrix(0, 4, 5); trip3detects=matrix(0, 4, 5);
trip4detects=matrix(0, 4, 5)
trip5detects=matrix(0, 4, 5); trip6detects=matrix(0, 4, 5); trip7detects=matrix(0, 4, 5)
trip8detects=matrix(0, 4, 5); trip9detects=matrix(0, 4, 5); trip10detects=matrix(0, 4, 5)

for(x in 1:4){
  detct=x*.2            # if 1:4 then detctprob ranges from 0.2 to 0.8

for(y in stoprulemin:stoprulemax){
  stoprule=y              #loops from stoprulemin to stoprulemax

tripdetects=matrix(0, trips, reps)
for (r in 1:reps) {       # Replicates loop starts here

###Pond Sampling
detected = matrix(0, columns, rows)
detection = array(0, c(trips, sum(pondscape)))
status <-rep(c(0),sum(pondscape))
z=1

for (m in 1:trips) {       #beginning of loops through sampling trips

###Select order of ponds for survey
if (m==1) {
  pondsurv=sample(sum(pondscape),sum(pondscape))     #creates random list of all ponds by
pondID for first survey
}
else {

  #randomly orders ponds after ranking by proximity for subsequent surveys
  pondsurv<- c(sample(P1), sample(P2), sample(P3), sample(P4), sample(PP1), sample(PP2),
sample(PP3), sample(PP4), sample(PPP1), sample(PPP2), sample(PPP3), sample(PPP4))
  pondsurv<-pondsurv[pondsurv>0]        #removes '0' placeholders in P1, P2, etc.
}

###Sample ponds for presence of salamanders
for (p in 1:pondssamp){               #begin loop of ponds per trip
     if (runif(1)<=detct) {                         #binomial prob compared to detection prob
        if (pondsocc[which(pondid==pondsurv[p], arr.ind=TRUE)]==1) {   #if pond occupied and
detected
          detection[m,pondsurv[p]]=1
          status[pondsurv[p]]=1
          detected[which(pondid==pondsurv[p], arr.ind=TRUE)]=1
        }
       else {
       detection[m,pondsurv[p]]=-1                          #pond occupied but not detected
```

```
        if (sum(detection[,pondsurv[p]])==(status[pondsurv[p]]-1)*(stoprule)) {
        status[pondsurv[p]]=status[pondsurv[p]]-1
        }
      }
    }
    else {                                              #for unoccupied ponds
      detection[m,pondsurv[p]]=-1                              #automatic nondetect
        if (sum(detection[,pondsurv[p]])==(status[pondsurv[p]]-1)*(stoprule)) {
        status[pondsurv[p]]=status[pondsurv[p]]-1
        }
    }
}                                 #end loop of ponds sampled per trip

###Calculate proximity of ALL cells to detected ponds for sampling decisions
proximity = matrix(0, columns, rows)
  #vectors of pond groups based on proximity and status
  P1<-c(0); P2<-c(0); P3<-c(0); P4<-c(0); PP1<-c(0); PP2<-c(0); PP3<-c(0); PP4<-c(0); PPP1<-
c(0); PPP2<-c(0); PPP3<-c(0); PPP4<-c(0)

  for (i in 1:sum(pondscape)){                #these steps revised in v7 for faster operation
    q<-which(pondid==i, arr.ind=TRUE)
        if (detected[q]>0){
        proximity[q] = 99
        }
        else {
          if (sum(detected[(q[1]-2):(q[1]+2),(q[2]-2):(q[2]+2)])>0){
          proximity[q] = .8                #this value not used in calculations except to rank by
proximity
          }                          #proximity to occupied pond is assigned 1 of 3 values (.8, .6, or .4)
          else  {
            if (sum(detected[(q[1]-5):(q[1]+5),(q[2]-5):(q[2]+5)])>0){
            proximity[q] = .6            #ditto
            }
            else  {
              if (sum(detected[(q[1]-8):(q[1]+8),(q[2]-8):(q[2]+8)])>0){
              proximity[q] = .4          #ditto
   } } } }
  }

for (i in 1:sum(pondscape)){
  if (proximity[which(pondid==i, arr.ind=TRUE)]==0.8) {
    if (status[i]==0) {
      P1<- c(P1,i)        #all ponds that meet the criteria for this proximity score are listed in P1.
    }
      else {
        if (status[i]==-1) {
```

```
      PP1<- c(PP1,i)
      }
      else {
        PPP1<- c(PPP1,i)
      }
    }
  }
else {
  if (proximity[which(pondid==i, arr.ind=TRUE)]==0.6) {
    if (status[i]==0) {
    P2<- c(P2,i)
    }                     #ditto
    else {
      if (status[i]==-1) {
      PP2<- c(PP2,i)
      }
      else {
        PPP2<- c(PPP2,i)
      }
    }
  }
  else {
    if (proximity[which(pondid==i, arr.ind=TRUE)]==0.4) {
      if (status[i]==0) {
      P3<- c(P3,i)
      }                   #ditto
    else {
      if (status[i]==-1) {
      PP3<- c(PP3,i)
      }
      else {
        PPP3<- c(PPP3,i)
      }
    }
  }
    else {
      if (proximity[which(pondid==i, arr.ind=TRUE)]==0) {
        if (status[i]==0) {
        P4<- c(P4,i)
        }                 #ditto
    else {
      if (status[i]==-1) {
      PP4<- c(PP4,i)
      }
      else {
        PPP4<- c(PPP4,i)
```

```
      }
     }
    }
  } } } }
  tripdetects[m,r]=sum(detection[m,]>0)
}                              #end of 'm' loop for trips

#OUTPUT

#SUMMARY STATS

pondssmpld=sum(colSums(abs(detection[1:trips,]))>0)  #total ponds sampled (no. of non-zeros in last row)
dd=sum(detected)                    #no. of occupied ponds sampled and detected
ee=sum(colSums(abs(detection[1:trips,]))*occsvector>0)-dd     #no. of occupied ponds sampled but not detected
cc=sum(occsvector)-(dd+ee)              #no. of occupied ponds not sampled
aa=sum(colSums(abs(detection[1:trips,]))==0)-cc    #no. of unoccupied ponds not sampled
bb=sum(pondscape)-sum(occsvector)-aa      #no. of unoccupied ponds sampled
#gg=sum(detection[passes,]*xx==-.01) #no. of occupied given up on after repeated non-detects

#colSums(abs(detection[1:trips,]<0))  #number of undetects for each pond
#colSums(abs(detection[1:trips,]>0))  #number of detects for each pond
#sum(colSums(abs(detection[1:trips,]<0))>0) #number of ponds with at least 1 undetect
#sum(colSums(abs(detection[1:trips,]))==0)  #number of unsampled ponds

#COMPILED STATS FOR MULTIPLE REPS
ttlpondssmpld[r]=pondssmpld
aaa[r]=aa
bbb[r]=bb
ccc[r]=cc
ddd[r]=dd
eee[r]=ee

#SUMMARIZING DETECTS PER TRIP
trip1detects[x,y]=mean(tripdetects[1,]); trip2detects[x,y]=mean(tripdetects[2,]);
trip3detects[x,y]=mean(tripdetects[3,])
trip4detects[x,y]=mean(tripdetects[4,]); trip5detects[x,y]=mean(tripdetects[5,]);
trip6detects[x,y]=mean(tripdetects[6,])
trip7detects[x,y]=mean(tripdetects[7,]); trip8detects[x,y]=mean(tripdetects[8,]);
trip9detects[x,y]=mean(tripdetects[9,])
trip10detects[x,y]=mean(tripdetects[10,])

cat("rep",r,'complete','\n')
}                              #end of 'r' loop for reps
```

```
SUMttlpondssmpld[x,y]= mean(ttlpondssmpld)
AVGaaa[x,y]=mean(aaa)
STDaaa[x,y]=sd(aaa)
AVGbbb[x,y]=mean(bbb)
STDbbb[x,y]=sd(bbb)
AVGccc[x,y]=mean(ccc)
STDccc[x,y]=sd(ccc)
AVGddd[x,y]=mean(ddd)
STDddd[x,y]=sd(ddd)
AVGeee[x,y]=mean(eee)
STDeee[x,y]=sd(eee)

cat('y=',y,'\n')
}                          #end of 'y' loop for stoprule
cat('x=',x,'\n')
}                          #end of 'x' loop for detctprob

print('total ponds sampled');print(SUMttlpondssmpld)
print('AVGaaa');print(AVGaaa)
print('AVGbbb');print(AVGbbb)
print('AVGccc');print(AVGccc)
print('AVGddd');print(AVGddd)
print('AVGeee');print(AVGeee)
print('STDaaa');print(STDaaa)
print('STDbbb');print(STDbbb)
print('STDccc');print(STDccc)
print('STDddd');print(STDddd)
print('STDeee');print(STDeee)
print('avg detects per trip - detection prob by stoprule')
print(trip1detects); print(trip2detects); print(trip3detects); print(trip4detects); print(trip5detects)
print(trip6detects); print(trip7detects); print(trip8detects); print(trip9detects); print(trip10detects)
```

A.6 KNOXVILLE NEWS SENTINEL ARTICLE

Salamander science

OAK RIDGE NATIONAL LABORATORY

Oak Ridge National Laboratory wildlife biologist Neil Giffen, right, and project technician Kara Ravenscroft study flatwoods salamanders in a wetland at Fort Stewart, Ga. The lab is studying ways to better locate endangered species such as the flatwoods salamander.

ORNL researcher's persistence

A search for rare salamanders in south Georgia is a small project with big implications.

Environmental scientists at Oak Ridge National Laboratory hope that tracking a hard-to-find population of flatwoods salamanders (ambystoma cingulatum) will provide a roadmap for future efforts to locate threatened or endangered species.

FRANK MUNGER
In the Lab

In the United States, the dark-gray salamanders — 3 to 5 inches as adults — are found in Florida, South Carolina and Georgia.

According to information provided by ORNL:

"The adults lay their eggs in wetland depressions in the late fall. With winter rains, the ponds rise and the eggs hatch in December, January and February. Larval development lasts through May. The larvae then undergo metamorphosis and leave the ponds as terrestrial juveniles. Juvenile and adult salamanders may move 400 or more yards from ponds and burrow in old crayfish holes and other crevices in soggy pine flatwoods."

The focal point of the lab's research effort is Fort Stewart, a sprawling 280,000-acre Army base west of Savannah, Ga.

Even though hundreds of seasonal ponds on the military base have been identified as potential habitat for the rare salamanders, none of the little critters had been seen around there in four years.

A 100-year drought began in 1999 and lasted for a couple of years, and there were questions about whether the Fort Stewart population had vanished.

At least that was the case until April 21.

That's when Kara Ravenscroft, an ORNL technician, found and trapped the first flatwoods salamander.

What was the key?

Persistence, for one thing. By sampling over and over and over again, Ravenscroft extended the known date of larval flatwoods salamanders in Fort Stewart's ponds by two to three weeks. It was well beyond the date at which biologists thought they would leave the ponds.

A larval flatwoods salamander.

Because of dry conditions in December and February, the wet-weather ponds didn't rise as normal.

"The standard thought was the eggs wouldn't survive as long as they did," Mark Bevelhimer, an environmental researcher at ORNL, said of the salamander search.

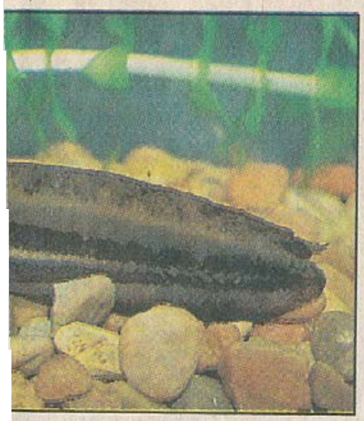
Following the initial discovery, the research team identified 36 more of the rare salamanders in one pond but none in sev-

pays off in search for rare species

eral other ponds that have supported populations in the past.

ORNL is participating in a strategic research program that's sponsored by multiple federal agencies, including the Defense Department, the Department of Energy, and the Environmental Protection Agency.

"We got money to help develop some new sampling techniques and better ways to find rare species," Bevelhimer said. "This is sort of our test case. I think we were able to put forth a little more effort and be a little more persistent."



Staff members at Fort Stewart, like their counterparts at federal installations everywhere in the United States, are required to identify species listed on the endangered list and try to protect them. With funds in short supply, the research program was a welcome boost to that effort.

According to the South Carolina Wildlife Federation, the flatwoods salamander has declined throughout the region in recent years.

"The historic loss of our southern longleaf pine forests, due to conversion to loblolly pine plantations and agriculture, has diminished the salamander's terrestrial habitat," the federation said in a report on its Web site. "Poor management of existing longleaf habitat, primarily the exclusion of periodic fire, has resulted in a degradation of remaining longleaf habitat."

The species also has been hurt as temporary wetlands — used as breeding grounds — have been drained and converted to other uses in the Southeast, the federation said.

Preservation of the flatwoods salamanders at Fort Stewart has benefited from efforts already under way there to save the red cockaded woodpecker, which resides in the same type of areas, Bevelhimer said.

The Army stages regular burns in the savannah to mimic natural fires and to maintain the habitat, the scientist said.

The ORNL team hopes to establish models that can be used on future projects, whether it involves tracking and identifying salamanders or just about anything else that's hard to find in nature.

"We're trying to come up with ways to use our brains a little bit ... to be in the right places at the right time and give ourselves the best odds of finding them," Bevelhimer said. "We have to establish a sort of baseline of what's effective."

There are laws that protect threatened and endangered species, but, beyond that, it's just the right thing to do, the Oak Ridge biologist said.

"We'd like to preserve everything. It's just good stewardship," Bevelhimer said. "If we let one species go, it can have a snowball effect. If you push back, you can slow the whole process."

Senior writer Frank Munger may be reached at 865-342-6329.

APPENDIX B. PUBLICATIONS

B.1 PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

B .2 FAWN ARTICLE

B.1 PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

Publications

Bevelhimer, M.S., D.J. Stevenson, N.R. Giffen, and K. Ravenscroft. (2008). Annual Surveys of Larval *Ambystoma Cingulatum* Reveal Large Differences in Dates of Pond Residency. Southeastern Naturalist 7:311-322.

Stevenson, D.J., and M.S. Bevelhimer. 2006. Fort Stewart Studies Rare Salamander. The FAWN (Publication of the National Fish & Wildlife Association) 24(3):15-16. (see Appendix B.2).

Fields, W.R., W.W. Hargrove, and M.S. Bevelhimer. (submitted to Journal of Wildlife Management). Testing Habitat Models for a Rare Salamander across Its Range.

Bevelhimer, M.S., W.R. Fields, and W.W. Hargrove. (in preparation). A Simulated Sampling Model for Evaluating Sample Design for a Rare Species.

Bevelhimer, M.S., N.R. Giffen, W.R. Fields, and D.J. Stevenson. (in preparation). Determining the Relationship between Catch Rate and Detection Probability for a Rare Salamander, *Ambystoma cingulatum*.

Published Technical Abstracts

"Needle in a Haystack: Sampling Design for Surveying Rare Species" – Oral presentation at Joint Services Environmental Management Conference – Denver, CO (May 5-8, 2008).

"Geographic Range Distribution and Habitat Modeling to Support Presence/Absence Sampling of a Rare Salamander at Fort Stewart, Georgia" – Poster presentation at Annual Meeting of Southeastern Partners in Amphibian and Reptile Conservation, Athens, GA (Feb 21-28, 2008).

"Finding a Needle in a Haystack: Tools for More Efficient Surveying of Rare Species" - Poster presentation at Partners in Environmental Technology Technical Symposium and Workshop in Washington, DC (December 2007).

"Finding a Needle in a Haystack: Tools for More Efficient Surveying of Rare Species" – Poster presentation at 2007 Sustaining Military Readiness Conference in Orlando, FL (July 30, 2007)

"The Effect of Local and Landscape Features on Wetland Amphibian Distributions" – Poster presentation at the International Association for Landscape Ecology meeting in Tuscon, AZ (April 9-13, 2007)

"Habitat Modeling at Multiple Spatial Scales to Support Sampling of a Rare Salamander" - Poster presentation at Partners in Environmental Technology Technical Symposium and Workshop in Washington, DC (November 29, 2006).

“Challenges in Documenting Rare Species Presence: Importance of Considering Rainfall Periodicity in Flatwoods Salamander Monitoring” - Poster presentation at Partners in Environmental Technology Technical Symposium and Workshop, Washington, DC, November 29-30, 2005.

“Minimizing Uncertainty in Presence/Absence Classification of Rare Salamander Populations” - Oral presentation at the SERDP sponsored Symposium and Workshop on Threatened, Endangered, and At-Risk Species on DoD and Adjacent Lands, Baltimore, MD (June 8, 2005).

B.2 FAWN ARTICLE

THE FAWN

"Dedicated to providing Natural Resources Management on Department of Defense lands in support of the Military Mission"

Volume XXIV | Number 3 | **October 2006**

Inside this Issue:

Now is the time to submit your articles on events or field studies taking place on your installation.

THE WILD SIDE

Rhys Evans – NMFWA President

In this newsletter, you'll find a reprint of two articles from BATS magazine, originally published by Bat Conservation International (BCI). I've been a member of BCI for more than a decade, I think. One thing I really like about BCI is that they only ask me for a donation twice per year, unlike so many other organizations that send 2-3 "urgent" letters asking for contributions every month.

When we asked for (and obtained) permission to reprint this article, their editor wrote "I was very pleased…to find that the military was so actively involved" in bat conservation. That also tells us that we can work a bit harder to get the word out about other good things we do on our installations. It's not a question of "if a tree falls in a forest…," but if we don't tell people about the good things we're doing, there is certainly somewhat less of an impact.

In my last "Wild Side," I told you that I'd like to take a few minutes to introduce you to the new members of your Board of Directors. I take that back…I'd like to make it even better. I've asked each of the officers and directors to write a very brief biography, so we're no longer just names and e-mail addresses (at least for those of you who don't come to our annual meetings and drink beer with us…). Though it's a work in progress, check out what we've started on our website (click on "Board Members" at the top menu).

Mike Passmore is assembling a great program for 2007 in Portland. We've got a banquet site locked up and several top-notch Technical Sessions coming your way. One really cool detail is that the great Portland light rail system will get you from the airport to our conference

Continued on next page

Fort Stewart Studies Rare Salamander

By Dirk J. Stevenson and Mark Bevelhimer

Larval flatwoods salamander. Photo by Dirk J. Stevenson

Neil Giffin and Kara Ravenscroft check aquatic traps at a known flatwoods salamander breeding pond. Photo by Dirk J. Stevenson

Sampling methods for an uncommon and protected salamander are being studied at Fort Stewart, GA by researchers based at the Oak Ridge National Laboratory in Tennessee.

Adults of the flatwoods salamander, *Ambystoma cingulatum*, a medium-sized (to 5 inches in total length) species in the Mole Salamander family (Ambystomatidae), spend the majority of their lives underground. Endemic to pine savannahs and flatwoods of the southeastern Coastal Plain, this amphibian was federally listed as "Threatened" in 1999 due to habitat loss. Adults of this salamander travel to isolated, depressional wetlands forested with pond cypress and black gum to breed during the autumn-early winter. Their aquatic larvae, strikingly patterned with yellow and brown, subsequently develop in these ponds and metamorphose during March-May.

Fort Stewart (280,000 ac) contains an abundance of suitable habitat for this rare salamander. The extensive, high-quality longleaf pine flatwoods landscapes scattered over the installation are literally pocked with these "cypress-gum ponds". Over 1,200 of these "wet-weather", temporary pond-type wetlands (most from less than 1 ac to 3 ac in size) have been mapped for the base, and approximately 500 of these were identified by a leading flatwoods salamander authority as "potential breeding habitat" for the species. And to date, 21 flatwoods salamander breeding sites have been documented on Fort Stewart. The vast amount of good habitat is due in large part to the installation's active habitat management and prescribed-fire program, since the flatwoods salamander favors open-canopied, grassy breeding sites and terrestrial flatwoods habitats maintained by frequent fire. Since 1992, Fort Stewart has prescribe-burned an average of 104,000 ac/year, with an average of 40,000 ac/year burned during each growing season (March-September) over this period. Of course, open-canopied, good-visibility, and navigable pine forests are also favored by the military for training purposes.

Due to a variety of reasons, discerning salamander presence at potential breeding ponds, and even finding salamanders at known sites, is often difficult. Because locating the strongly fossorial (= burrowing) adults is well-nigh impossible without using costly and extremely labor-intensive drift fence techniques, flatwoods salamander surveyors throughout the range of the species (South Carolina-Georgia-Florida) have traditionally sampled by dipnetting for larvae. But, if low population densities of larvae are present at breeding ponds (habitats that tend to be densely choked with sedges and grasses and hard to dipnet thoroughly) then the species presence may easily be overlooked. It is conceivable that an occupied pond could be sampled for several years before the first larval salamander is found.

This is where the team from Oak Ridge comes in. Aquatic ecologists Dr. Mark Bevelhimer and habitat modeler Dr. Bill Hargrove of the Oak Ridge National Laboratory's Environmental Sciences Division were awarded a 3-year grant via the Strategic Environmental Research and Development Program (SERDP) to study field sampling techniques. By combining field sampling and computer analysis, the researchers hope to develop guidelines for more efficient sampling.

Thus far, the team has spent two springs sampling ponds with various types of small traps and dipnets to find out which methods are most effective at capturing flatwoods salamanders and to better understand what

Continued on next page

Continued from previous page

mixture of aquatic organisms (other amphibians and fish) are indicators of an environment that is conducive to flatwoods salamander breeding. This evaluation also includes a thorough analysis of the different types of plants that are in and around the ponds.

To date the team has sampled over 70 ponds at Fort Stewart, including most of the 21 where flatwoods salamanders had been observed previously, and found larvae in only one pond. However, that's better than anyone else in the state of Georgia has done! Flatwoods salamanders found in 2005 and 2006 at Fort Stewart are the only ones found in the state in the past six years. Three years of severe drought from 1999-2002 prevented any reproduction during those years and the population seems to be struggling to recover.

One goal of the project is to be able to predict which ponds are most likely to support breeding so that survey efforts can be concentrated in those areas. Because there are so many factors to consider, the researchers will be depending on some sophisticated computer models to determine which environmental factors, such as plant species, water quality, and predator abundance, correspond best with salamander presence or absence.

Not only is the research team trying to figure out where to sample, but also when to sample. The presence of the salamanders in the ponds is closely tied to winter and spring rains and the filling of the temporary ponds. In Georgia, flatwoods salamander larvae typically metamorphose and leave the ponds in March or April. Because the first months of 2005 were unusually dry, the salamanders didn't hatch till late in the spring. The result of the late hatching was that flatwoods salamanders were collected in the pond that year through the end of May, the latest pond residency ever documented.

Noted astronomer Carl Sagan once said, "Absence of evidence is not evidence of absence." Not being able to find flatwoods salamanders doesn't mean they are not in a particular habitat, but with information gained during this study, biologists from Fort Stewart and Oak Ridge hope to be able to increase the confidence with which they proclaim a particular pond to be likely breeding habitat or not. Greater certainty in these predictions will benefit the conservation of this rare species without hampering the important military mission of Fort Stewart.

A dry cypress pond typical of habitat where flatwoods salamanders breed during the wet season.
Photo by Dirk J. Stevenson

DEADLINES FOR SUBMITTING ARTICLES TO EDITOR FOR UPCOMING FAWN EDITIONS:

31 DECEMBER for JANUARY 2007

Articles on installation activities may be submitted through your respective Regional Directors (Eastern Region—Tim Beatty and Tessa Martin-Bashore; Western Region —Kirsten Christopherson and Sandy Baldwin) or directly to me, Rick Lance (richard.lance@nmfwa.org)

Electronic files should preferably be in MS Word®, Times New Roman-12 font. One or two clear pictures (JPG format) may be beneficial (more may be negotiated) and will be used if space allows.

APPENDIX C. GENERAL RECOMMENDATIONS FOR LARVAL FLATWOODS SALAMANDER SAMPLING

GENERAL RECOMMENDATIONS

FOR SAMPLING FLATWOODS SALAMANDERS

A three-year study funded by the Department of Defense's Strategic Environmental Research and Development Program (SERDP) on sampling strategies for flatwoods salamanders (*Ambystoma cingulatum*) resulted in several recommendations for future survey work for flatwoods salamanders across its range. These recommendations address why, how, when, where, what, and how often to survey in order to maximize the detection of flatwoods salamander larvae in breeding ponds. To learn more about the details behind these recommendations please see the final report for this study which is available at the SERDP website http://www.serdp.org/Research/SI-Natural-Resources-Management.cfm or the U.S. Department of Energy (DOE) Information Bridge web site http://www.osti.gov/bridge as ORNL Tech. Rpt. TM-2008/192.

Why?

The reason for sampling flatwoods salamanders (or other species of concern) varies depending on management or research needs. Common reasons for sampling potential breeding ponds include: determining presence of larvae; documenting long-term population trends; and exploring habitat requirements and limitations. The sampling approach one chooses to use depends on the reason for sampling, the number of potential ponds, and prior knowledge of pond occupancy. For example, the sampling approach for a site with few ponds with a high proportion of occupancy based on prior sampling would be quite different than that for a site with many ponds and little knowledge of prior occupancy, especially if funding is limited.

How?

The study results suggest that dipnetting is the preferred method of sampling for larval flatwoods salamanders at least when compared to other methods tested, metal and plastic minnow traps and wood-framed, total water column, funnel traps. It is possible that more effective traps could be developed that take advantage of specific flatwoods salamander behavior, but the traps tested (which are commonly used to sample for flatwoods salamander larvae) are not as effective as dipnetting based on capture rate or cost (labor plus materials).

When?

Capture of larvae is most successful when larvae are most abundant and large enough to be retained in dipnets and easily seen amongst the debris that is collected during each sweep. The period of highest susceptibility to capture is primarily during the second and third month after hatching. Date of hatching can best be estimated as being within a day or two of significant rises in pond water elevation in December through February. Hatching can occur as late as March in Georgia and perhaps elsewhere, and multiple hatching dates within a pond are likely unless the pond fills entirely over a few days time.

Since the level of pond filling that is necessary to result in hatching is not easily determined, one or two sentinel ponds that are dependable flatwoods salamander breeding sites should be regularly monitored from December through March (or until larvae are found) as an indicator of area-wide reproductive success or pond residency. Evidence of hatching in a representative and reliable pond (or ponds) can then be used to dictate the best time to sample a large number of ponds. Hatching and metamorphosis typically occur later at more northerly latitudes, but non-simultaneous pond filling could conceivably reverse this general trend. Based on the late residency observed in Georgia in 2005, it is possible that detection of late hatching larvae might not be susceptible to capture until April in northern latitudes if adequate water levels persist in the ponds.

Where?

When faced with more ponds to sample than available resources can manage, the choice of which ponds to sample should be made in a systematic way. The results of computer simulations performed during the study suggest that if there is a known factor that correlates with salamander occupancy (e.g., proximity to a documented breeding pond, habitat similarity, presence or absence of predators/competitors, or food availability), then that factor or suite of factors should be used to develop an adaptive sampling plan. In an area where previous sampling has been unable for one reason or another to produce a list of likely breeding ponds, the first round of sampling should be from a randomly selected subset of all potential ponds. If larvae are detected in some ponds and there is a common factor among them (such as habitat quality) then that factor should be used in the selection of ponds for subsequent rounds of sampling. If on the other hand there is no known common thread among inhabited ponds, then choice of ponds should continue to be randomly selected.

In the SERDP study, several statistical models were tested for their ability to predict larval flatwoods salamander presence based on pond habitat and landscape characteristics and historical occurrence. Although the particular habitat features that predict occupancy vary from place to place, it appears that habitat along with proximity to known ponds should be considered when selecting ponds for sampling. If more than one statistical model or different suites of habitat variables are used then it is advisable to use these results to generate an index that combines the results of multiple models into a single score. In this way multiple analyses can be used in combination to generate a priority list of ponds for future sampling starting with those that ranked most similar to ponds with known occupancy.

Within a pond, the location of larvae appears to be more closely related to the location of particular vegetation types than to any geographic feature of the pond such as distance from edge of pond, water depth, or other physical characteristics. There are specific vegetation types that seasoned experts recognize as likely habitat for flatwoods salamander larvae and these are easily conveyed to inexperienced netters. These typically include primarily emergent grasses and other plants with slim vertical stalks or blades such as wiregrass (*Aristida* species), beakrush (*Rhynchospora* species), and hatpin (*Eriocaulon* species).

What?

When sampling to determine larval presence, there is other information that can be collected at the same time that will provide useful data for understanding many of the questions surrounding flatwoods salamander presence and detection. Survey time should be closely monitored with periodic stops (e.g., every 5 min) to record the number of larvae captured for each netter. If possible, noting the exact time of each capture is even better. These data can be used to estimate detection probability at a particular pond. Long-term data on sampling success in an area can be used to calculate local occupancy rates. Estimates of detection probability and occupancy can be used to establish a level of confidence in declaring that a pond is unoccupied after repeated unsuccessful surveys. Snout-vent and total lengths should be recorded so that estimates of pond residency (hatch date and time to metamorphosis) can be calculated.

Pond characteristics also provide useful information for interpreting survey data. Pond elevation or pond depth should be measured every trip to a pond for a better understanding of pond hydroperiod. Although collecting enough data for a habitat analysis takes a lot of effort, some observations should be made at least annually at each pond visited. Any noticeable changes in species composition or abundance should be noted for the pond and surrounding upland, especially if any exotic or invasive species appear or increase in abundance. A more detailed habitat analysis once every 4 to 5 years at select known ponds would provide valuable data for evaluating long-term changes in pond plant communities that might significantly impact the ponds' ability to support salamander reproduction.

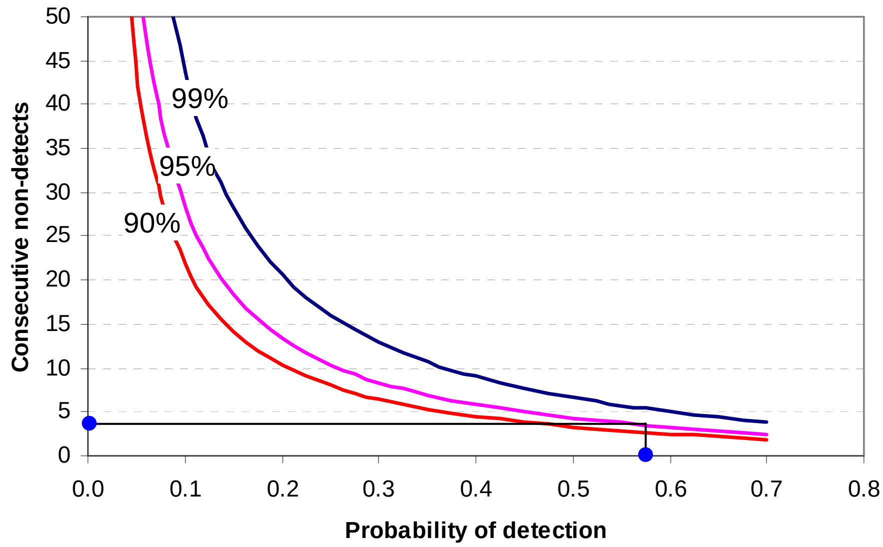
How often?

Because the detection probability of flatwoods salamander larvae can be low when abundance is low and as a result of their cryptic nature, it often takes more than one sampling visit to a pond to detect occupancy when present. Given that resources for a monitoring program are usually limited, efficient distribution of effort is therefore of utmost important. How many times a pond should be sampled following repeated non-detects and how to distribute sampling effort among ponds are important questions.

For individual ponds, the decision to quit sampling after repeated non-detects should be based on the level of confidence one desires in declaring a pond is unoccupied and on the probability of detecting at least one salamander during a sampling event. During the SERDP study, detection probabilities were estimated for several ponds based on the proportion of successful 5-minute sampling periods in occupied ponds. Detection probabilities are largely a function of density and ranged from 0.07 to 1.0 for a single netter during 5 minutes of sampling (or 0.58 to 1.0 for an hour of sampling). One can calculate the number of consecutive non-detects (x) needed at a particular pond to be certain with an established level of confidence that the pond is unoccupied using the following equation:

$$x = \ln(\alpha) / \ln(1 - p)$$

where α is the confidence level (i.e., α=0.05 equates to 95% confidence) and *p* is the probability of detection. Note that this calculation presumes that each trip would represent the same duration of sampling as that upon which the detection probability is based. The same information can be derived from the following figure.



This figure shows the number of consecutive trips necessary to achieve 90, 95, and 99% confidence of absence for a range of detection probabilities. For example, using the lowest probability of detection calculated from the SERDP study (0.58 for an hour of sampling), it would require 4 consecutive 1-hr surveys without finding a larva to be 95% confident that a pond is unoccupied.

Since detection probability for a pond of unknown occupancy cannot be known with certainty, there are several approaches for using the method. If larvae are detected at nearby ponds then one might choose to use the average detection probability for unknown ponds. Alternatively, it might be more conservative to use the lowest detection probability observed at other ponds for the unknown ponds. Once a detection probability is chosen, the number of surveys necessary to achieve a specified level of confidence can be selected from the figure or calculated with the equation.

The high number of larvae found in several ponds in near proximity at St Marks NWR in 2007 suggests that meta-populations respond similarly and annual variation in larvae density is similar among ponds. Given this observation, an efficient sampling approach for many locations might be to distribute effort unevenly among years, expending as much as possible in years when conditions and sampling at dependable ponds suggest that it might be a banner year for reproduction.

Sampling populations is a critical task for the conservation and management of flatwoods salamanders. Careful planning should identify how larval surveys should be conducted to meet management objectives. While sampling a rare species may appear daunting, careful study design and consideration of detection probabilities may greatly improve confidence in interpreting survey results.